

PROTOCOL REPORT

Natural Attenuation of Perchlorate in Groundwater: Processes, Tools
and Monitoring Techniques

ESTCP Project ER-0428

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ACRONYMS AND ABBREVIATIONS

AFB	Air Force Base
AFCEE	Air Force Center for Environmental Excellence (now: Air Force Center for Engineering and the Environment)
ASTM	American Society for Testing and Materials
ATK	Alliance Techsystems
bgs	below ground surface
Br ⁻	bromide ion
CaDHS	California Department of Health Services
CD	chlorite dismutase
Cl ⁻	chloride ion
cld	chlorite dismutase gene
cm ³	cubic centimeters
ClO ₄ ⁻	perchlorate
CO ₂	carbon dioxide
DNA	deoxyribonucleic acid
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DoD	Department of Defense
Eh	measure of oxidation-reduction potential
EOS [®]	Emulsified (edible) Oil Substrate
ESTCP	Environmental Security Technology Certification Program
FDA	Food and Drug Administration
Fe(II)	ferrous iron
Fe(III)	ferric iron
ft	feet
IC	ion chromatography
ITRC	Interstate Technology and Regulatory Council
LC	liquid chromatography
LC/MS	Liquid Chromatography/Mass Spectroscopy
LHAAP	Longhorn Army Ammunition Plant
LMTA	Little Mountain Texas Annex
MBT	molecular biological tool
MCL	maximum contaminant level
MCT	matrix conductivity threshold
ml	milliliters
MRL	minimum reporting level

ACRONYMS AND ABBREVIATIONS (continued)

mRNA	messenger RNA
MTBE	methyltert-butyl ether
mV	millivolts
MNA	monitored natural attenuation
MPN	most probable number
MS	mass spectroscopy
MW	monitoring well
NDCEE	National Defense Center for Environmental Excellence
NO ₃ ⁻	nitrate
NSWC	Naval Surface Warfare Center
NWIRP	Naval Weapons Industrial Reserve Plant
ORP	oxidation-reduction potential
OSW	Office of Solid Waste
OSWER	Office of Solid Waste and Emergency Response
<i>pqr</i>	perchlorate reductase gene
PCR	polymerase chain reaction
ppb	parts per billion
ppm	parts per million
PVC	polyvinyl chloride
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
SERDP	Strategic Environmental Research and Development Program
SWMU	Solid Waste Management Unit
TCE	trichloroethene
TDS	total dissolved solids
TOC	total organic carbon
USEPA	United States Environmental Protection Agency
VOA	volatile organic analysis
VOC	volatile organic compound

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1.0 EXECUTIVE SUMMARY

Sources of perchlorate can be both natural and anthropogenic, and the extent of perchlorate in the environment is becoming more widely acknowledged. The fate and transport of this inorganic contaminant are still being studied. Because of the potential health risks associated with its consumption, there is regulatory pressure to establish meaningful and realistic goals for cleanup. Depending on the state, regulatory limits for perchlorate in groundwater range from 1 to 24.5 µg/L. Monitored natural attenuation (MNA) is one of several new technologies being evaluated for effectiveness in remediating perchlorate in groundwater.

Acceptance of MNA typically requires multiple lines of evidence. For perchlorate, biodegradation is especially important because it is not readily sorbed, volatilized, or abiotically degraded. Analytical methods are available to monitor the concentration of perchlorate in the environment with high sensitivity and selectivity, geochemical tests can indicate whether ambient conditions are conducive to perchlorate biodegradation, and molecular biological tools (MBT) are being developed to monitor the activity and sustainability of perchlorate-reducing bacterial populations. When properly applied, MNA of perchlorate can be protective of human and environmental health.

This guidance document presents a systematic tiered approach to determine the potential for natural attenuation of perchlorate in groundwater. The three tiers of evidence include 1) plume stability, 2) geochemical indicators, and 3) biological indicators (USEPA, 1999).

Tier 1: Plume Stability and Geometry. Historical data can be used to delineate the extent of the contamination and determine the fate of contaminants of concern. With a properly designed monitor well network, trends in the data can successfully illustrate plume geometry and stability. Ideally, one should show that the contaminant plume is stable or retreating. A stable or shrinking perchlorate plume would indicate that attenuation processes are removing perchlorate from the groundwater at least as fast as perchlorate is released from the source area. The simplest tools available include visual isopleth maps and concentration trend analysis versus time. Relatively simple statistical techniques can also be used to evaluate plume stability including regression analyses, the Mann-Whitney U Test, the Mann-Kendall Test, and center of mass calculations. The challenge at all sites is to understand the inherent temporal and spatial variability so the data obtained are meaningful and can be correctly interpreted. This can require extensive monitoring with data acquisition over long time periods. Tier 1 monitoring to evaluate changes in the spatial and temporal distribution of perchlorate can offer the first line of evidence that perchlorate is naturally attenuating.

Tier 2: Biogeochemical Conditions. The collection of site-specific biogeochemical information is the best understood and most widely employed step to evaluate the potential for MNA of perchlorate. Research has shown that many microorganisms have the genetic capability to degrade perchlorate, and perchlorate-reducing bacteria are present in numerous and disparate environments. Many of the same parameters important for natural attenuation of chlorinated solvents are equally important for assessing the potential for perchlorate to biodegrade. Optimal conditions for MNA of perchlorate include low dissolved oxygen (DO) (i.e., anaerobic or microaerophilic conditions), a reducing environment with a negative oxidation-reduction

potential (ORP), pH between 5 and 8, nitrate concentrations less than 5 mg/L, and total organic carbon (TOC) concentrations greater than 2 mg/L. Elevated methane and reduced iron are also indirect indicators of a favorable environment. The practitioner must keep in mind that these groundwater parameters serve as indicators of favorable conditions for natural attenuation of perchlorate.

Tier 3: Microbiological Indicators. For situations where additional lines of evidence are required, Tier 3 offers laboratory and field tests that provide both indirect and direct evidence of perchlorate biodegradation. The perchlorate reductase (*pcr*) gene catalyzes the conversion of perchlorate to chloride and chlorite. The chlorite dismutase (*cld*) gene reduces chlorite to chloride and oxygen. Qualitative polymerase chain reaction (PCR) and quantitative PCR (qPCR), performed on microbial deoxyribonucleic acid (DNA) extracted from site matrices, can provide a sensitive, rapid approach to evaluate the molecular potential for perchlorate biodegradation to occur. When performed on ribonucleic acid (RNA) from the same population, these methods are useful as direct measures of ongoing bioactivity.

Microcosms and bench-scale column studies can be used to demonstrate that natural attenuation is occurring and to estimate the rate and extent of perchlorate biodegradation. However, these studies are time-consuming and expensive to implement and should not be employed until a good understanding of site conditions has been achieved through site investigation activities (i.e., Tier 1 and Tier 2). If laboratory studies are conducted, laboratory biodegradation rate derived from these studies should be used with care since differences between field and laboratory conditions can lead to non-representative results.

Newer field methods, including installation of in situ columns and stable isotope monitoring, can be used where there is expectation that anaerobic biodegradation of perchlorate is occurring. In situ columns isolate an intact column of soil and groundwater from the rest of the aquifer and can be used to monitor the rate of perchlorate biodegradation over time within a controlled but natural environment. Isotopic ratios of chlorine and oxygen atoms in perchlorate ($^{35}\text{Cl}/^{37}\text{Cl}$ and $^{16}\text{O}/^{18}\text{O}$) provide another tool to measure the extent of perchlorate degradation. Stable isotope analysis provides a method for distinguishing biotic from abiotic attenuation since microorganisms often preferentially use lighter isotopes in their metabolic processes. As a contaminant is degraded, the isotopic composition of the remaining material becomes progressively heavier. If there is clear evidence of isotopic shifts, the extent of perchlorate degradation can be estimated using the fractionation factor.

The guidance provided in this document is meant to assist in monitoring the fate of perchlorate in the environment and provide a systematic approach to evaluate the potential for perchlorate MNA. The weight of evidence obtained through this tiered process can be used to identify sites where MNA is a safe and effective approach for managing perchlorate-impacted groundwater.

2.0 INTRODUCTION

2.1 PURPOSE OF THIS DOCUMENT

The purpose of this document is to provide users with information on 1) fate, transport and transformation of perchlorate in different geochemical environments; 2) emerging and/or specialized technologies for evaluating perchlorate attenuation in groundwater; and 3) a tiered approach for evaluating the MNA of perchlorate. This protocol does not provide a step-by-step guide to MNA of perchlorate. Instead, it presents several procedures to aid the user in determining if natural attenuation processes are sufficient to meet remedial objectives in a reasonable time frame while protecting human health and the environment. Before implementing a study to assess MNA of perchlorate, users should be generally familiar with previously developed protocols for assessing the MNA of petroleum hydrocarbons and chlorinated solvents. Depending on site-specific conditions and regulatory factors, the user can implement some or all of these procedures to develop a weight-of-evidence case for MNA as a viable remedial strategy for perchlorate-impacted groundwater.

2.2 BACKGROUND

The acceptance of natural attenuation as a groundwater remedy has grown rapidly over the last decade. In the mid to late 1980s and into the 1990s, numerous researchers and practitioners in the field of environmental engineering noticed that solute plumes were not migrating nearly as far as predicted based on commonly held tenants of solute behavior in the subsurface. In fact, many solute plumes were stable or receding. Concurrently, research studies were conducted at several universities to elucidate the causes for the greater-than-predicted solute degradation. These studies determined that biodegradation, both aerobic and anaerobic, was significantly more important than originally thought. In the early to mid 1990s, several groups, most notably the Air Force Center for Environmental Excellence (AFCEE)¹, began to take the knowledge gained in the laboratory and apply it at the field scale. Large amounts of solute and biogeochemical data were collected and used to evaluate plume behavior. It was found that many, if not most, of the solute plumes were stable or receding and that biological mechanisms were largely responsible for keeping the solutes from migrating downgradient with the advective flow of groundwater. Based on the information gained from these studies, several technical protocols for evaluating the natural attenuation of common contaminants were published. Although these documents are still valid as a framework for evaluating the natural attenuation of many common contaminants in the subsurface, perchlorate does not fall neatly into the groups of contaminants discussed in the commonly cited protocols. The approach presented in this document references framework protocols for the reader's use but details the mechanisms specific to the natural attenuation of perchlorate.

2.3 MONITORED NATURAL ATTENUATION

The U.S. Environmental Protection Agency (USEPA) defines MNA as a "knowledge-based" remedy that relies on natural processes of contaminant attenuation to achieve site-specific remediation requirements within a reasonable time frame as compared to other more active

¹ Name changed to Air Force Center for Engineering and the Environment in 2007.

methods (USEPA, 1997). These natural processes include a variety of physical, chemical, and biological methods such as biodegradation, dilution, sorption, and volatilization that under favorable conditions reduce the mass, toxicity, mobility, volume, or concentration of contaminants without human intervention (USEPA, 1997). Like enhanced bioremediation, MNA requires an in-depth understanding of the microbiology, chemistry, and hydrogeology of the environment under consideration (Interstate Technology and Regulatory Council [ITRC], 2002). Unlike enhanced bioremediation, MNA does not involve the active anthropogenic manipulation of in situ conditions. Instead, an evaluation of natural attenuation typically involves an assessment of 1) site geology and hydrogeology, 2) the nature and disposition of the contaminants, and 3) the efficacy of degradation mechanisms (typically biological) in removing contaminant mass from the system. Taken together, the assessment of these parameters allows the investigator to determine if naturally occurring processes are capable of achieving remediation goals in a reasonable period of time (ITRC, 2002). In addition, an evaluation of natural attenuation typically takes into account the biodegradation rates and the suitability of the natural biogeochemical conditions to support or sustain attenuation.

MNA has evolved as an accepted remedial approach for petroleum hydrocarbons (ASTM, 2004) and chlorinated solvent groundwater plumes (Wiedemeier et al., 1998). Although less widely accepted, MNA has also been applied for other contaminants, such as methyl-*tert*-butyl ether (Wilson et al., 2005), wood preservatives (Stroo et al., 1997), and nitroaromatic explosives (Pennington et al., 1999). Numerous guidance documents and technical protocols are available to assist users with evaluating and implementing MNA for various contaminants. These documents include:

- American Society for Testing and Materials (ASTM), 2004. *Standard Guide for Remediation of Ground Water by Natural Attenuation at Petroleum Release Sites*. Standard E 1943-98 (Reapproved 2004), American Society for Testing and Materials, West Conshohocken, Pennsylvania.
- Wiedemeier, T.H., 1995. *Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Groundwater*. Air Force Center for Environmental Excellence.
- Wiedemeier, T.H. et al., 1998. *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater*. USEPA., EPA/600/R-98/128.
- Wiedemeier, T.H. et al., 2006. *Designing Monitoring Programs to Effectively Evaluate the Performance of Natural Attenuation*. Air Force Center for Environmental Excellence.
- Kennedy, L. et al., 2000. *Aqueous and Mineral Intrinsic Bioremediation Assessment (AMIBA) Protocol*. Air Force Center for Environmental Excellence.
- AFCEE, 1999. *Methyl tert-Butyl Ether (MTBE) – Its Movement and Fate in the Environment and Potential for Natural Attenuation*. Air Force Center for Environmental Excellence.

- Wilson, J.T., 2000. *Natural Attenuation of MTBE in the Subsurface under Methanogenic Conditions*. USEPA, EPA/600/R-00/006.
- Pennington, J.C. et al., 1999. *Draft Protocol for Evaluating, Selecting, and Implementing Monitored Natural Attenuation at Explosives-Contaminated Sites*. Technical Report EL-99-10, U.S. Army Engineer Research Center. September 1999.
- American Petroleum Institute, 2007. *Protocol for Evaluating the Natural Attenuation of MTBE in Groundwater*.

Although MNA has become a widely accepted approach for remediation of all of these contaminants, MNA of perchlorate is still in a relatively immature stage of development.

2.4 EPA POLICY ON MONITORED NATURAL ATTENUATION

Office of Solid Waste and Emergency Response (OSWER) Directive 9200.4-17 (USEPA, 1997) establishes EPA's expectations for application of MNA. In general, the appropriateness of MNA should be supported by site-specific data and analysis used in conjunction with a conceptual site model of contaminant fate and transport. Additionally, once those data have been collected and the model developed, the potential successfulness of MNA as a remediation strategy should be evaluated by collecting more detailed site-specific information about:

- 1) Historical data on groundwater and/or soil chemistry to demonstrate a decrease in contaminant mass and/or concentration
- 2) Hydrogeologic and geochemical data to demonstrate indirectly the types of natural attenuation (specifically degradation) occurring at the site
- 3) Data from field or microcosm studies which directly demonstrate the occurrence of specific natural attenuation processes at the site.

Generally, the historical data (number 1 above) are accompanied by data identifying the natural attenuation processes (number 2 above) unless the EPA or other overseeing regulatory agency deems otherwise. Data from microcosm studies (number 3 above) are required when information provided by items 1 and/or 2 are not conclusive enough to be self-supporting. As with other remediation methods, long-term monitoring and documentation are required to ensure that the risk is being reduced to acceptable levels (USEPA, 1997). Table 1 summarizes the EPA's policy on MNA.

Table 1. Summary of the EPA MNA Policy.
 (Pennington et al., 1999)

General Elements in the Evaluation of Monitored Natural Attenuation	Related Factors, Issues, and Actions
Role of MNA in the remedy selection process	<ul style="list-style-type: none"> • May be an appropriate alternative under a limited set of circumstances • May be evaluated and compared with other viable remedies during the study phases leading to the selection of remedy • May be cautiously evaluated as a sole remedy • May be evaluated as a component of a total remedy that includes engineered remedial measures • May be evaluated as a follow-on to engineered remediation • Must not be considered a default or presumptive remedy
General requirements for the selection of MNA as a remedy	<ul style="list-style-type: none"> • Must meet all relevant remedy selection criteria • Must be fully protective of human health and the environment • Must meet site remediation objectives within a reasonable time frame compared with other methods • Must be supported by detailed site-specific information that demonstrates its efficacy • Must evaluate all contaminants that represent an actual or potential threat to human health or the environment • Must include opportunities for public involvement to both educate and gather feedback from interested parties
Requirements for the demonstration of the effectiveness of MNA through site characterization	<ul style="list-style-type: none"> • Site characterization will involve the collection and development of data and conduct of analyses to demonstrate that natural attenuation can meet the remedial action objectives. At a minimum, the following actions will be required: <ul style="list-style-type: none"> ○ Collect data to define nature and distribution of contamination sources ○ Collect data and conduct analyses to define the extent of the groundwater plume and potential impacts on receptors • Other data and information required will be dependent upon site-specific characteristics, the nature of the contaminants, and the natural attenuation process(es) being evaluated • Data quality must be adequate, levels of confidence on attenuation rates documented, and sensitivity analyses performed to determine dependence of calculated remediation time frames on uncertainties in rate constants and other factors
Requirements for the evaluation of the efficacy of MNA through site-specific lines of evidence	<ul style="list-style-type: none"> • The evaluation of efficacy may include the collection and evaluation of the following data and information: <ul style="list-style-type: none"> ○ Historical groundwater and soil data that clearly demonstrate declining contaminant concentrations and/or masses ○ Hydrogeologic or geochemical data that can indirectly demonstrate the mechanisms involved in natural attenuation at the site and the rate at which contaminant reductions occur ○ Data from field or microcosm studies that demonstrate the occurrence of a natural attenuation process and its ability to effect contaminant reductions (particularly through degradation)
Requirements for the implementation of MNA	<ul style="list-style-type: none"> • Source control and performance monitoring should be fundamental components of the remedy • Institutional controls may be necessary • Performance monitoring should continue as long as contamination remains above cleanup levels • Remedies employing natural attenuation should include evaluation of need for one or more contingency remedies

Adapted from EPA, 1999.

The advantages and disadvantages of MNA compared to other remediation processes were identified in OSWER Directive 9200.4-17 (USEPA, 1997) as follows:

The **advantages** of MNA remedies include:

- As with any in situ process, generation of lesser volume of remediation wastes reduced potential for cross-media transfer of contaminants commonly associated with ex situ treatment, and reduced risk of human exposure to contaminated media
- Less intrusion as few surface structures are required
- Potential for application to all or part of a given site, depending on site conditions and cleanup objectives
- Use in conjunction with, or as a follow-up to, other (active) remedial measures
- Lower overall remediation costs than those associated with active remediation.

The potential **disadvantages** of MNA include:

- Longer time frames may be required to achieve remediation objectives, compared to active remediation.
- Site characterization may be more complex and costly.
- Toxicity of transformation products may exceed that of the parent compound.
- Long-term monitoring will generally be necessary.
- Institutional controls may be necessary to ensure long-term protectiveness.
- Potential exists for continued contamination migration, and/or cross-media transfer of contaminants.
- Hydrologic and geochemical conditions amenable to natural attenuation are likely to change over time and could result in renewed mobility of previously stabilized contaminants, adversely impacting remedial effectiveness.
- More extensive education and outreach efforts may be required in order to gain public acceptance of MNA.

In general, each state has its own guidance on the use of MNA. Therefore, the appropriate state regulatory agency should be consulted to determine their current policy.

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3.0 FATE AND TRANSPORT OF PERCHLORATE IN GROUNDWATER

3.1 PURPOSE OF THIS SECTION

Groundwater and surface water contaminated with perchlorate have become a major environmental issue for the U.S. Department of Defense (DoD) due to the use, release, and/or disposal of solid rocket fuel and munitions containing ammonium perchlorate. Perchlorate is highly mobile and soluble and sorbs poorly to most aquifer material. It can persist for decades under aerobic conditions. As a consequence, discharge of perchlorate to the environment has resulted in extensive contamination of surface and groundwater supplies. In 1997, the California Department of Health Services (CaDHS) developed an analytical technique to detect perchlorate concentrations as low as 4 µg/L (Motzer, 2001). Since then, perchlorate concentrations at or above 4 µg/L have been found in the surface and groundwaters in over 35 states (USEPA, 2005b). In the western United States, more than 15 million people consume water with some level of perchlorate.

The human health concern from perchlorate is the inhibition of iodide uptake resulting from decreased thyroid hormone output, which can disrupt metabolism (USEPA, 2005b). Additionally, environmental health concerns are associated with the uptake of perchlorate in food crops such as lettuce and milk (Kirk et al., 2003; USEPA, 2005b; Jackson et al., 2005; Renner, 2006). Currently, there is no federal cleanup standard for perchlorate in groundwater or soil (USEPA, 2005a; ENS, 2006). In January 2006, the USEPA issued “Assessment Guidance for Perchlorate” identifying 24.5 µg/L as the recommended value “to be considered” and preliminary remediation goal for perchlorate (USEPA, 2006). In 2006, California proposed a maximum contaminant level (MCL) for perchlorate in drinking water of 6 parts per billion (ppb) (CaDHS, 2006). In July 2007, Massachusetts became the first state in the nation to promulgate drinking water and wastewater standards for perchlorate, adopting a standard of 2 ppb and requiring most public water systems to regularly test for perchlorate (ENS, 2006). Several states, but not all, have identified perchlorate advisory levels. These range in concentration from 1.0 to 52 µg/L. Remediation practitioners should consult with local regulatory authorities before adopting specific cleanup goals for their site.

In recent years, an extensive body of information has been developed demonstrating that a large and diverse population of microorganisms can degrade perchlorate to chloride and oxygen (Coates et al., 1999; Coates and Pollock, 2003). Perchlorate-reducing organisms are widespread in the environment (Coates et al., 1999; Logan, 2001), including pristine and hydrocarbon-contaminated soils, aquatic sediments, and industrial and agricultural waste sludges (Gingras and Batista, 2002). Perchlorate biodegradation can occur under strict anaerobic conditions as well as facultative anaerobic conditions, i.e., in mixed aerobic/anaerobic environments. This metabolic versatility suggests that environments exist that can support a variety of perchlorate-reducing microbial populations and that perchlorate may naturally degrade at some sites without active human intervention. Because there is a strong potential for MNA of perchlorate where site conditions are appropriate, identifying lines of evidence that suggest which sites are amenable to perchlorate MNA is highly important. The purpose of this section is to identify the sources and

characteristics of perchlorate so that appropriate lines of evidence can be selected to evaluate perchlorate MNA.

3.2 SOURCES OF PERCHLORATE

Perchlorate (ClO_4^-) is composed of a chloride atom bonded to four oxygen atoms (Figure 1).

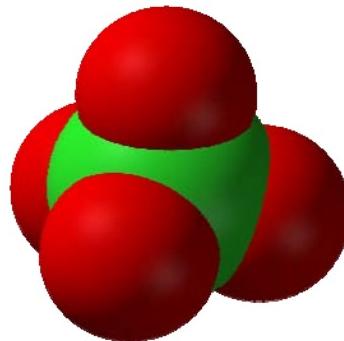


Figure 1. Perchlorate Anion.
(ITRC, 2005)

Perchlorate is usually found as the anion component of a salt and is released when the solid salts of ammonium, sodium, or potassium perchlorate and perchloric acid dissolve in water (Motzer, 2001).

3.2.1 Anthropogenic Sources

Perchlorate has been manufactured since the 1890s and is most commonly found as a manufactured compound (ITRC, 2005). The most common compounds² are ammonium perchlorate, sodium perchlorate, potassium perchlorate, and perchloric acid. Of these, in the United States, approximately 90% of the production is of ammonium perchlorate (Xu et al., 2003). Ammonium perchlorate is used as an oxidizing agent for solid propellant rockets and missiles. Other common uses for perchlorate are indicated in Table 2. Based on these uses, the presence of co-contaminants such as volatile organic compounds (VOC), halogenated solvents, explosive compounds (e.g., trinitrotoluene [TNT]; hexahydro-1,3,5-trinitro-1,3,5-trizine [RDX]; and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine [HMX]), nitrate, and sulfate are also found with perchlorate (ITRC, 2002).

² See Appendix C of ITRC (2005) for a listing of additional, less common manufactured perchlorate compounds.

Table 2. Uses of Perchlorate.
(ITRC, 2005)

Chemical and Electrical Uses	Explosive and Propellant Uses	Miscellaneous Uses
Cathodic protection systems	Military devices	Steel plate bonding
Brine separation	Geoseismic devices	Li-ion batteries
Chlorate/chlorite manufacturing	Chemical cutter	Enamel paints
Cloud seeding	Ordnance	Fertilizer
Dielectric for transformers	Tracer bullets	Laundry bleach
Electroplating	Solid rocket motor	Pharmaceutical
	Rocket motor	Diagnosis/treatment
	Airbags	Pool sanitizer
	Ejection seats	
	Fireworks	

3.2.2 Natural Sources

Perchlorate can be naturally occurring, but its exact origins are not known with certainty. A current theory suggests that in a process similar to nitrate formation in the atmosphere, chloride reacts with ozone to form perchlorate. Additionally, natural sources of perchlorate have been limited to arid regions and are often found as evaporite minerals in such natural materials as bromine, borates, gypsum, and nitrogen compounds (ITRC, 2005). In nature, the highest concentrations of perchlorate are found in Chilean caliche and potash ores (ITRC, 2005). Recent research has used isotopic fractionation methods to characterize perchlorate found in different locations worldwide to determine differences between naturally occurring and anthropogenic sources (Sturchio et al., 2006; Bohlke et al., 2005; Hatzinger et al., 2007).

There are other projects that are continuing to examine the natural occurrence of perchlorate. These include two Environmental Security Technology Certification Program (ESTCP)-funded projects: Evaluation of Alternative Causes of Widespread, Low Concentration Perchlorate Impacts to Groundwater (ER-1429) and Identification and Characterization of Natural Sources of Perchlorate (ER-1435). The reader is encouraged to review these documents for additional information on this topic.

3.3 PHYSICAL AND CHEMICAL PROPERTIES OF PERCHLORATE

The properties of common perchlorate salts are shown in Table 3.

Table 3. Properties of Perchlorate Compounds.
(ITRC, 2005)

Properties	Ammonium perchlorate (NH_4ClO_4)	Potassium perchlorate (KClO_4)	Sodium perchlorate (NaClO_4)	Perchloric acid (HClO_4)
CAS#	7790-98-9	7778-74-7	7601-89-0	7601-90-3
Molecular weight	117.49	138.55	122.44	100.47
Color/form	White orthorhombic crystal	Colorless orthorhombic crystal or white crystalline powder	White orthorhombic deliquescent crystal	Colorless oily liquid
Taste/odor	Odorless	Slightly salty	Odorless	Odorless
Density/specific gravity	1.95 g/cubic centimeters (cm^3)	2.53 g/ cm^3	2.52 g/ cm^3	1.67 g/ cm^3
Solubility	200 g/L water @ 25°C	15 g/L water @ 25°C	2096 g/L water @ 25°C	miscible in cold water
Sorption capacity	Very low	Very low	Very low	Very low
Volatility	Nonvolatile	Nonvolatile	Nonvolatile	Volatile
Octanol/ H_2O partition coefficient ($\log K_{ow}$)	-5.84	-7.18	-7.18	-4.63
Vapor density (air = 1)	No information	4.8	No information	3.5
pH	5.5 to 6.5	6.0 to 8.5	7.0	Highly acidic

Although dissolved perchlorate tends to be associated with groundwater, it is important to recognize that other factors such as density and aquifer surface charge may also influence the fate and transport of perchlorate within the aquifer. For example, solid perchlorate salts like ammonium perchlorate and highly concentrated solutions of perchlorate, known as brine, can behave similarly to dense non-aqueous phase liquid (DNAPL) when released into an aquifer system. As such, the perchlorate tends to sink through the water column until the mass reaches a low permeability confining layer (Motzer, 2001) where it persists, causing secondary or recurring perchlorate contamination (ITRC, 2002). Additionally, Motzer (2001) references studies showing that the ammonium cation derived from ammonium perchlorate brines will displace all other exchangeable cations in the soil matrix when it is retarded in soil. One study of silt and clay samples from the saturated zone and/or aquitard with high cation concentrations suggests that high concentrations of ammonium would remain in the soil and provide forensic clues regarding the source of perchlorate and plume history (Motzer, 2001).

3.4 ATTENUATION PROCESSES

Several sources can be referenced for details and definitions of fate and transport mechanisms controlling environmental contaminants (Wiedemeier et al., 1995; Wiedemeier et al., 1998; Wiedemeier et al., 1999; ITRC, 2002). In general, these processes are controlled by both the contaminant's physical and chemical properties as well as the properties of the media through which the compound travels. The movement of perchlorate in soil is dependent on the amount of water present since perchlorate does not readily bond to soil particles (ITRC, 2005). Both abiotic and biologic processes affect the transformation of groundwater contaminants. However,

abiotic reactions are much slower than biologic ones and are typically assumed to be negligible for perchlorate (ITRC, 2002). Table 4 summarizes the important attenuation processes.

Table 4. Summary of Important Subsurface Processes Acting on Perchlorate
(Wiedemeier et al., 1999; ITRC, 2002)

Process	Description	Dependencies	Effect
Advection	Movement of solute by bulk groundwater movement	Dependent on aquifer properties such as hydraulic conductivity, effective porosity, and hydraulic gradient Independent of contaminant properties	Main mechanism driving contaminant movement in the subsurface
Dispersion	Fluid mixing due to groundwater movement and aquifer heterogeneities	Dependent on aquifer characteristics and scale of observation Independent of contaminant properties	Causes longitudinal, transverse, and vertical spreading of the plume Reduces solute concentration
Diffusion	Spreading and dilution of contaminant due to molecular diffusion	Dependent on contaminant properties and concentration gradient Described by Fick's Laws	Diffusion of contaminant from areas of relatively high concentration to areas of relatively low concentration Generally unimportant at most groundwater flow velocities
Sorption	Reaction between aquifer matrix and solute	Dependent on aquifer matrix properties (organic carbon and clay mineral content, bulk density, specific surface area, and porosity) and contaminant properties (solubility, hydrophobicity, octanol-water partitioning coefficient)	Tends to reduce solute transport rate and remove solutes from the groundwater via sorption to the aquifer matrix
Infiltration (Simple dilution)	Infiltration of water from the surface to the subsurface	Dependent upon the aquifer matrix properties, depth to groundwater, and climate.	Causes dilution of the contaminant plume and replenishes electron acceptor concentrations, especially DO
Biodegradation	Microbially-mediated oxidation-reduction reactions that transform perchlorate to chloride and oxygen	Dependent on groundwater geochemistry, microbial population, and contaminant properties Perchlorate biodegradable under anaerobic and facultative anaerobic conditions	Results in complete mineralization of perchlorate to chloride and oxygen

3.4.1 Advection and Dispersion

Two mechanisms for contaminant movement in groundwater are advection and hydrodynamic dispersion. Advection is the movement of contaminants in the direction of and at the velocity of

groundwater flow. Advection usually provides a greater contribution to mobility where the contaminant is highly soluble in water. Assuming that advection is the dominant mechanism, contaminant movement can be estimated using Darcy's Law.

Hydrodynamic dispersion is another mechanism for contaminant movement. Hydrodynamic dispersion is the sum of both molecular diffusion and mechanical mixing and results in the outward spread of or dilution of a dissolved contaminant apart from the flow of contaminants due to advection alone. Molecular diffusion is a result of the thermal-kinetic energy of the solute particles and is typically important at low groundwater velocities while mechanical dispersion is the spreading of molecules as a result of interactions between the advective movement of the contaminant and the porous structure of the medium. For perchlorate, these processes are known as nondestructive mechanisms because they result in the reduction of concentration, but not the total mass. In general, as longitudinal dispersivity increases, the maximum concentration decreases and the time to reach steady state increases (Newell et al., 2002). Figure 2 illustrates a one-dimensional breakthrough curve showing plug flow resulting from advection and dispersion. This spreading occurs both in the direction of groundwater flow, longitudinal dispersion, as well as perpendicular to groundwater flow, transverse dispersion.

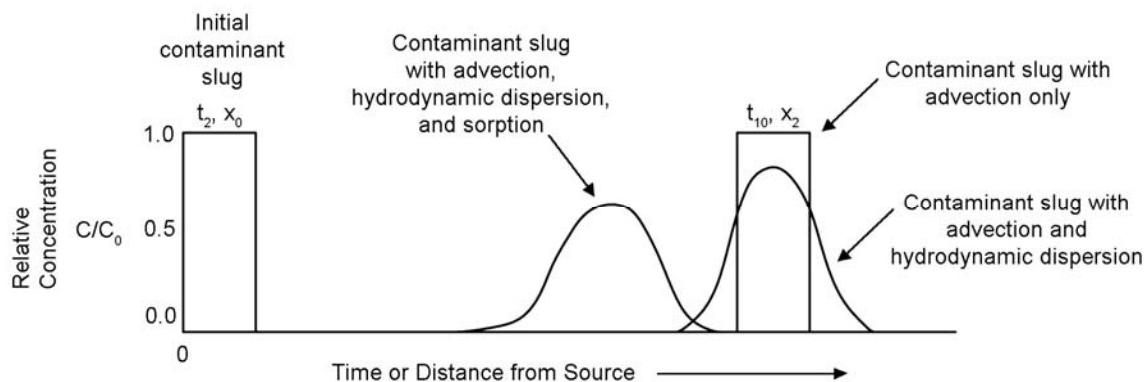


Figure 2. Plug Flow Resulting from Advection Only and Advection and Dispersion.
(Wiedemeier et al., 1999)

There are several forms of perchlorate salts that are manufactured in large amounts. In general, perchlorate salts are very soluble and once released to the environment will leach downward through the vadose zone into the groundwater with water infiltrating from the surface. In dilute concentrations, the leached perchlorate will travel with the average velocity of the groundwater. However, dispersion will often cause the leading edge of the contaminant to move somewhat faster than the average groundwater velocity (ITRC, 2005).

Dispersion can facilitate biodegradation by spreading the perchlorate beyond that anticipated by advection alone (ASTM, 2004).

3.4.2 Sorption to Aquifer Material

Sorption is the process where dissolved contaminants absorb onto soil surfaces (ASTM, 2004) and, therefore, play an important role in fate and transport. The chemical properties of the contaminant determine how strongly it will absorb onto soil surfaces. For example, the

concentrations of a contaminant in groundwater will be reduced if a dissolved contaminant absorbs strongly to the soil, and becomes part of the aquifer matrix.

Sorption is considered a nondestructive mechanism because it only affects a contaminant's mobility and concentration and does not reduce the total mass present in the aquifer. It is generally assumed that anions such as bromide (Br^-), chloride (Cl^-), nitrate (NO_3^-) and perchlorate (ClO_4^-) do not sorb to aquifer materials because the most common clay minerals have a net negative charge. However, it is possible for the charge of aquifer materials to be altered. For example, some minerals and poorly crystalline iron oxides become more positively charged at low pH (Brooks et al., 1998) and do not bind as strongly to anions present in the aquifer. Under these conditions, movement of Br^- , Cl^- , and NO_3^- can be somewhat retarded by anion exchange occurring on the positively charged surfaces (Brooks et al., 1998; Clay et al., 2004). This suggests that ClO_4^- might also sorb to sediment surfaces under certain conditions and slow the movement of perchlorate within the aquifer system.

Perchlorate is generally assumed to sorb very weakly to soil surfaces and tends to migrate at essentially the same velocity as the groundwater (ITRC, 2002). Since many other groundwater contaminants (e.g., tetrachloroethene and trichloroethene [TCE]) sorb to soil surfaces, perchlorate may appear to advance more rapidly than other contaminants (Motzer, 2001).

3.4.3 Biodegradation Processes

Biodegradation is usually the most important destructive process when gaining acceptance of MNA as a remedial strategy for a site. The reduction of perchlorate is a thermodynamically favorable reaction, but the reaction is impeded by high activation energy. This makes perchlorate very chemically stable under normal groundwater and surface water conditions (Urbansky, 1999; NASA, 2006). Perchlorate has been shown to biodegrade via a three-step reduction mechanism in which perchlorate is sequentially reduced to chlorate, chlorite, and finally the innocuous end products, chloride and oxygen (Rikken et al., 1996). This pathway is illustrated in Figure 3. This enzyme-catalyzed reduction is very similar to biodenitrification. The perchlorate reducing microorganisms produce an enzyme that allows them to lower the activation energy for perchlorate reduction and to use perchlorate as an alternate electron acceptor for metabolism in place of oxygen or nitrate (NASA, 2006).

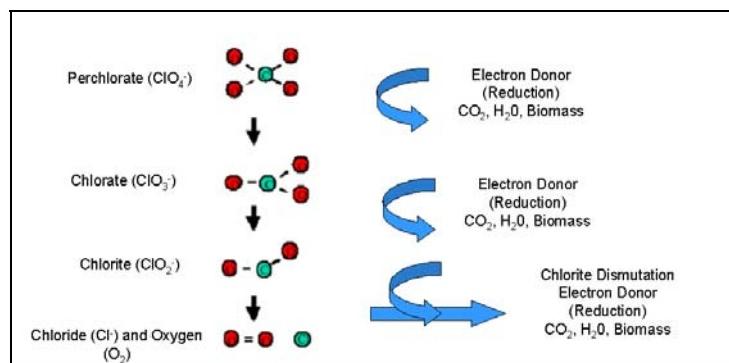


Figure 3. Perchlorate Biodegradation Pathway.

The first two steps in the breakdown of perchlorate are mediated by the same enzyme, *pcr*. The rate-limiting step in the three-step degradation process is the initial conversion of perchlorate to chlorate. Once converted, the chlorate is readily catalyzed to chloride by the same enzyme. Chlorite removal by the chlorite dismutase (CD) enzyme is the final step in perchlorate reduction (Xu and Logan, 2003). The specificity of the CD enzyme may be useful as an indicator of perchlorate biodegradation and, therefore, provide supporting evidence for MNA of perchlorate at certain sites. This is further discussed below.

An extensive body of information has been developed demonstrating that a wide diversity of microorganisms can degrade perchlorate to chloride and oxygen (Coates et al., 1999; Coates and Pollock, 2003; Xu and Logan, 2003). Perchlorate-reducing bacteria are widespread in the environment (Coates et al., 1999; Logan, 2001). Perchlorate-reducing bacteria are phylogenetically diverse with members in the alpha, beta, gamma, and epsilon subclasses of the Proteobacteria phylum (Coates and Achenbach, 2006). Experiments conducted by Coates et al. (1999) showed that perchlorate-reducing bacteria are completely oxidizing, gram-negative, and non-fermenting facultative anaerobes and are readily found in both contaminated and pristine environments. Most of these bacteria can also utilize nitrate as an electron acceptor suggesting that perchlorate reduction is distinct from nitrate reduction (Coates et al., 1999; Chaudhuri et al., 2002).

These organisms use either chlorate or perchlorate as a terminal electron acceptor and can use a variety of different organic substrates (e.g., acetate, propionate, lactate, and soybean oil) as electron donors (Herman and Frankenberger, 1998; Coates et al., 1999; Borden et al., 2006; Schaefer et al., 2007). Perchlorate biodegradation can occur under strict anaerobic conditions as well as facultative anaerobic and microaerophilic conditions (Rikken et al., 1996; Chaudhuri et al., 2002; Coates and Achenbach, 2006). When biodegradable organic substrates are present, the available DO will be consumed and there is a very high probability that perchlorate will biodegrade. Recent work has suggested that biological perchlorate reduction also can be linked to insoluble inorganic substrates. Ju et al. (2007) demonstrated that a chemolithotrophic enrichment culture could effectively couple the oxidation of elemental sulfur to sulfate with the reduction of perchlorate to chloride. The energy gained from the process was used for cell growth.

For in situ biodegradation to occur, other favorable geochemical conditions must also be present. For perchlorate reduction, potentially favorable geochemistry includes a pH between 6.5 and 7.5, an ORP between 0 and –100 millivolts (mV), low oxygen concentrations, and low nitrate levels (ITRC, 2002). In addition, studies and observations indicate that the presence of molybdenum may be required by perchlorate-reducing bacteria (Chaudhuri et al., 2002). The bioavailability of molybdenum is often a limiting nutrient in soils, especially acidic soils where adsorption reduces the availability of these salts at lower pHs (Chaudhuri et al., 2002). The implication of molybdenum dependence on perchlorate bioremediation strategies is important and may explain the persistence of perchlorate even in the presence of perchlorate-reducing bacteria (Chaudhuri et al., 2002). However, these different trace mineral requirements may only be required by laboratory media (Xu et al., 2003).

3.4.4 Abiotic Degradation Processes

By definition, abiotic processes are either chemical or physical. Typical abiotic mechanisms include the dilution, dispersion, and adsorption of chemical reactivity and result in the chemical reduction of perchlorate (Coleman et al., 2003; Sturchio et al., 2003). Since considerable energy (light, heat or another catalyst) is required to reduce the chlorine atom from a +7 oxidation state to a -1 state, chemical reduction of perchlorate is not seen in subsurface environments or in the laboratory even when the Eh (measure of ORP) of water is lowered to less than -200 mV (Motzer, 2001). It is presumed that dilution and precipitation have the most effect on the migration of perchlorate (Motzer, 2001; ITRC, 2002). Dilution causes the concentration of perchlorate to be significantly less the further away from the contamination source a sample is taken. While precipitation decreases the mobility of perchlorate, as a salt, perchlorate redissolves, is transported, and precipitates again in a continuous loop process.

3.4.5 Phytoremediation

Phytoremediation is a process, illustrated in Figure 4, that uses plants to remediate contaminated soil, surface water, and groundwater. Phytoremediation comprises different plant species and plant-mediated processes (Pilon-Smits, 2005). Many shrubs, trees, grasses, or wetland plants (and/or their associated microorganisms) can extract, sequester, transform, degrade, or transpire the contaminants (Nzengung and McCutcheon, 2003). Bench-scale studies confirm that plants can be effective for reducing concentrations of perchlorate-contaminated surface water, groundwater, and soils (Nzengung et al., 1999; Susarla et al., 1999, 2000; Nzengung et al., 2004; Nzengung and McCutcheon, 2003; Schnoor et al., 2002; Yifru and Nzengung, 2007b). Poplar trees and willow trees can degrade perchlorate completely to chloride (Xu et al., 2003).

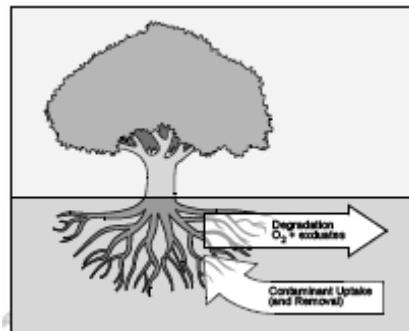


Figure 4. Phytoremediation Processes.
(USEPA, 2005b)

Perchlorate concentrations can be reduced by plants by two mechanisms: 1) uptake and phytodegradation, and 2) rhizodegradation (Nzengung et al., 1999; Nzengung et al., 2004). The amount of perchlorate taken up and phytoaccumulated by terrestrial and aquatic plants is influenced by several factors, including 1) the perchlorate concentration, 2) the type of plant species, and 3) the season. Rhizodegradation depends on the availability of dissolved organic carbon (DOC) or other electron donors and favorable biodegradation conditions in the rhizosphere. The concentration of available DOC is a limiting factor in the rhizodegradation of perchlorate (Mwegoha et al., 2007).

Rhizodegradation is more rapid and therefore a more desirable process for natural perchlorate biodegradation (Nzengung and McCutcheon, 2003; Yifru and Nzengung, 2007a and 2007b). Figure 5 shows the initial removal of perchlorate from water by slow plant uptake followed by rapid removal of perchlorate by rhizodegradation. The much higher rate of chloride formation is observed during the rhizodegradation phase. Chlorate formed as an intermediate degradation product of perchlorate does not persist in the root zone.

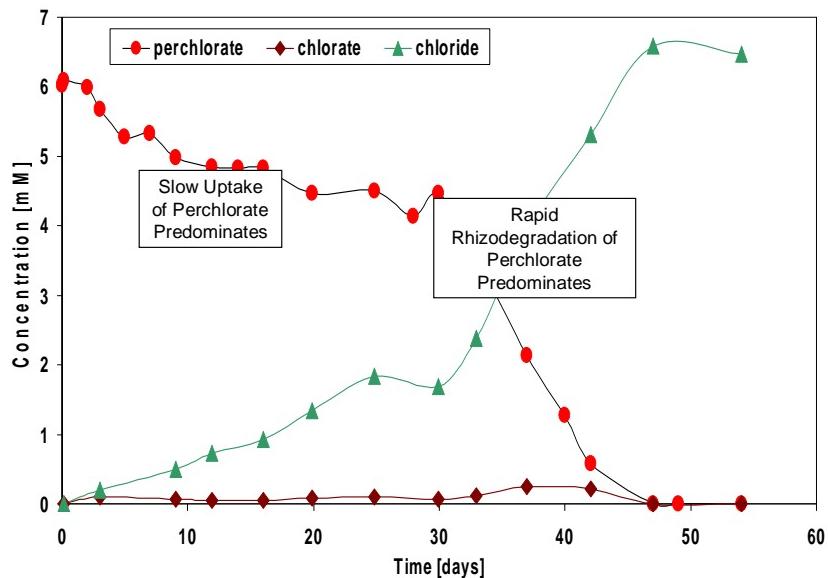


Figure 5. Relative Rate of Perchlorate Degradation by Phytoaccumulation Followed by Rhizodegradation.
(Nzengung et al., 1999)

Field studies have demonstrated that plant uptake and transformation can be important components of natural attenuation in wetlands impacted by perchlorate (Tan et al., 2004b, Krauter et al., 2005). Therefore, as perchlorate-contaminated groundwater migrates and comes in contact with plants, the opportunity for plant contribution to natural attenuation increases.

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4.0 TOOLS AND TECHNIQUES FOR EVALUATING PERCHLORATE ATTENUATION

4.1 PURPOSE

Section 2.0 provided a discussion of the sources, characteristics, fate and transport of perchlorate so that appropriate lines of evidence can be selected to evaluate perchlorate MNA. The purpose of this section is to inform the reader of various tools and techniques that can be used to evaluate perchlorate MNA.

The major portion of most perchlorate MNA investigations will include traditional tools to monitor spatial and temporal variations in contaminant concentration and geochemical indicators. The approach used for perchlorate will be very similar to that followed for chlorinated solvents. The reader should be familiar with these standard protocols first. Titles of several useful existing protocols are provided earlier in Section 2.3.

4.2 GEOCHEMICAL INDICATORS OF PERCHLORATE ATTENUATION

Geochemical data can be used to provide supporting evidence of natural perchlorate biodegradation. Groundwater geochemical data from across the site should be reviewed to identify whether favorable conditions for natural attenuation of perchlorate are present. The following subsections describe the geochemical conditions that are preferable for perchlorate biodegradation.

4.2.1 Dissolved Oxygen

DO is the most thermodynamically favored electron acceptor used by bacteria for the biodegradation of organic carbon, whether natural or anthropogenic (Weidemeier et al., 1998). Obligate anaerobic bacteria generally cannot function at DO concentrations greater than about 0.5 mg/L. In the presence of organic substrate, DO competes with perchlorate as an electron acceptor. Perchlorate-reducing bacteria can be strict anaerobes, microaerophiles or facultative anaerobes (Rikken et al., 1996; Chaudhuri et al., 2002) giving them the ability to grow either in the presence or absence of air, provided proper nutrients are available in the environment. The metabolic versatility of these organisms increases their sustainability in both contaminated and pristine environments.

Perchlorate degradation occurs when it is used as an electron acceptor in the presence of organic donor carbon. Although many perchlorate-reducing bacteria are versatile, DO concentrations greater than 2 mg/L are expected to inhibit perchlorate biodegradation, and DO concentrations less than 1 mg/L are expected to be more favorable for natural attenuation of perchlorate. The continued presence of DO will inhibit the potential for biodegradation of perchlorate. Conversely, in anaerobic environments, the opportunity for perchlorate to degrade in the role of electron acceptor improves.

4.2.2 Nitrate

Nitrate is a common co-contaminant in perchlorate contaminated waters. Most perchlorate-reducing bacteria are also denitrifiers and, as noted by Robertson et al. (2007), early work by Herman and Frankenberger (1999) suggested that nitrate-reduction (denitrification) and perchlorate reduction could occur under similar conditions and possibly using the same bacterial populations. Coates et al. (1999) suggested that most, but not all, perchlorate reducers can use nitrate as an electron acceptor, and Logan et al. (2001) noted the reverse, that some denitrifying bacteria are capable of perchlorate degradation (Logan et al., 2001).

There is evidence that perchlorate reduction can occur in the presence of alternate electron acceptors. Fixed-film bioreactor studies showed that simultaneous removal of DO, nitrate, and perchlorate could occur, but oxygen and nitrate needed to be completely removed before complete degradation of perchlorate could be observed (Min et al., 2004; Choi and Silverstein, 2008). Other studies have shown that the presence of nitrate usually decreases the rate of perchlorate reduction (Xu et al., 2003, Choi and Silverstein, 2008) but does not necessarily eliminate it. Nitrate has been shown to negatively regulate the production of CD and inhibit perchlorate reduction by the perchlorate-reducing bacterium *Dechlorosoma suillum* (Chaudhuri et al., 2002) now known as *Azospira suillum* (Coates and Achenbach, 2006; Tan and Reinhold-Hurek, 2003). However, in some studies, inhibitory effects of nitrate were not observed (Xu et al., 2003).

Tan et al. (2004a) tested microcosms to evaluate degradation kinetics of perchlorate in sediment and soils. They showed that perchlorate degradation was affected by organic substrate availability but not the concentration of nitrate. They concluded that more than one enzyme is involved and nitrate is not a competitive inhibitor of perchlorate enzyme activity. In further studies, Tan et al. (2004b) and Tan et al. (2005) found that lag time of perchlorate degradation in sediments increased with increased nitrate but decreased in the presence of higher substrate availability. They concluded that organic substrate availability would become the limiting factor under high electron acceptor conditions.

Studies to date have shown that the impact of nitrate concentrations on potential for perchlorate biodegradation is not absolute. There is no one concentration of nitrate below which perchlorate reduction is optimal. Ideally, low nitrate concentrations provide less competition with perchlorate as an electron acceptor in the environment. However, in presence of excess organic carbon, this may not be as important. Practitioners should consider nitrate status in the environment in conjunction with organic carbon availability and DO status when considering nitrate's potential impact on MNA of perchlorate.

4.2.3 Iron

Iron reduction is an anaerobic process in which ferric iron [Fe(III)] is reduced to ferrous iron [Fe(II)]. The reduced form of iron is soluble in water. Thus, an increase in dissolved iron can be an indicator of conditions favorable for perchlorate biodegradation. When dissolved iron concentrations are greater than 0.5 mg/L, this indicates anaerobic conditions with a high potential for perchlorate biodegradation.

4.2.4 Sulfate

Sulfate can also be used as an electron acceptor for anaerobic processes. But, sulfate reduction generally occurs after DO, nitrate, perchlorate (if present), and iron have been depleted in the microbiological treatment zone. Whereas sulfate concentration greater than 20 mg/L may cause competitive exclusion of anaerobic dehalorespiration of chlorinated solvents, the same is not true for perchlorate. In microcosms treated with 300 mg/L sulfate, there was no obvious effect on perchlorate biodegradation rates or lag time (Tan et al., 2004a).

4.2.5 Methane

Methane concentrations can be observed in the aquifer when more strongly reducing conditions are achieved, i.e., after depletion of oxygen, nitrate, perchlorate, and sulfate has occurred. During methanogenesis, acetate is split to form carbon dioxide (CO_2) and methane, or CO_2 is used as an electron acceptor and is reduced to methane (Weidemeier et al., 1998). Since perchlorate reduction is an anaerobic process, elevated concentrations of methane indicate favorable conditions for the natural attenuation of perchlorate.

4.2.6 Oxidation-Reduction Potential

Many aquifers contain bacteria that biodegrade contaminants via electron transfers (ITRC, 2002). The type of biotic chemical contaminant transformations that have the best possibility of occurring are indicated by the ORP, or redox, of the saturated zone (ITRC, 2002). Redox processes involve a change in valence state of the elements such that some are oxidized and others are reduced. The ORP of common chemicals and the delineation between aerobic and anaerobic processes are shown in Figure 6. Analysis for these chemicals provides information relative to the potential for and the types of bioremediation processes in the groundwater sample. Generally, the ORP is expressed in relation to the standard hydrogen electrode as Eh. Figure 6 shows the redox potential for different degradation processes.

ORP is a measure of the electron activity of the groundwater and an indicator of the relative tendency of a solution to accept or transfer electrons. The ORP of a groundwater system depends upon and influences rates of biodegradation (Weidemeier et al., 1998). The ORP of groundwater generally ranges from -400 mV to +800 mV. As illustrated in Figure 7, some processes operate most effectively within a prescribed range of ORP conditions.

Figure 7 illustrates the sequence of utilization of various electron acceptors found in a perchlorate-contaminated environment, graphically demonstrating why depletion of oxygen and nitrate concentrations must be accomplished before perchlorate can be degraded. It also illustrates why achieving ORP levels necessary for sulfate reduction and methanogenesis are not necessary or preferred for stimulating anaerobic perchlorate reduction. Thus, potentially favorable geochemistry for perchlorate reduction includes an ORP between 0 and -100 mV (ITRC, 2002).

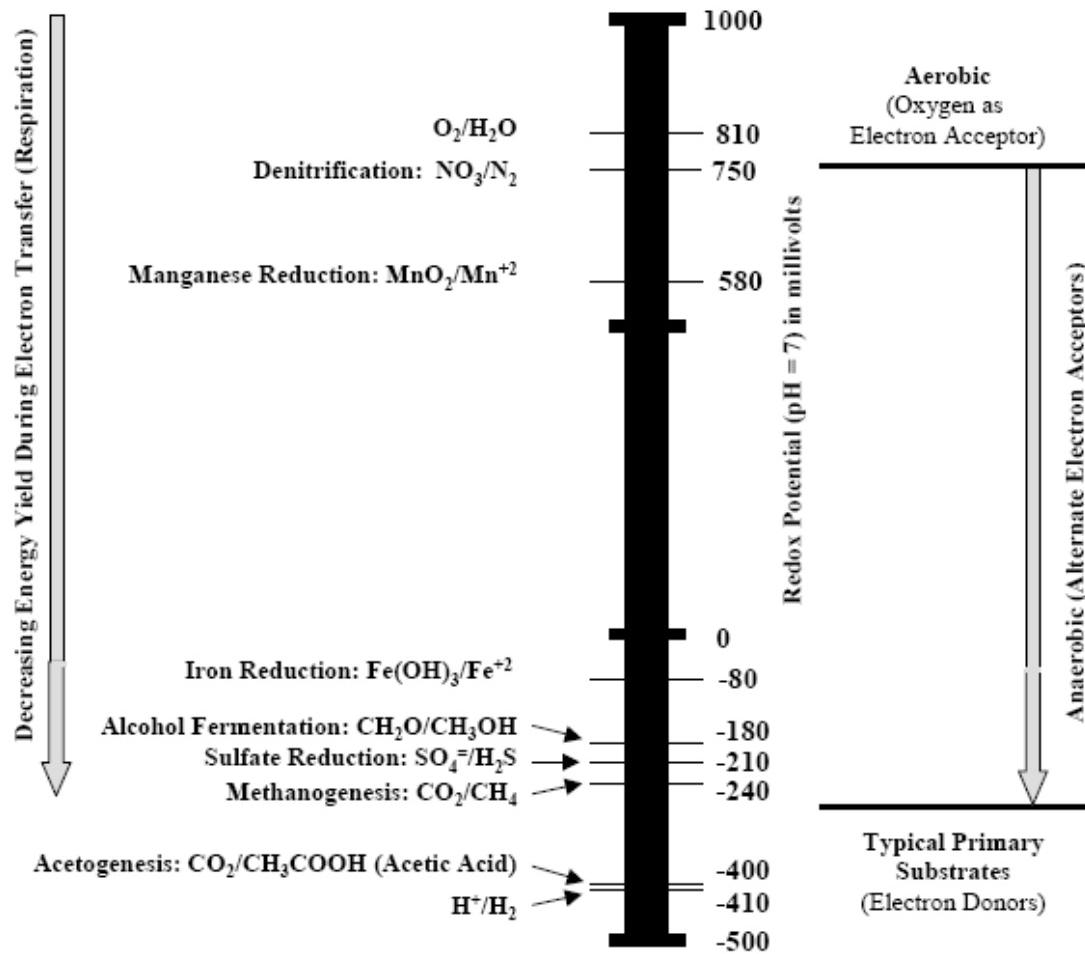


Figure 6. ORP of Common Chemical Species.
(ITRC, 2002)

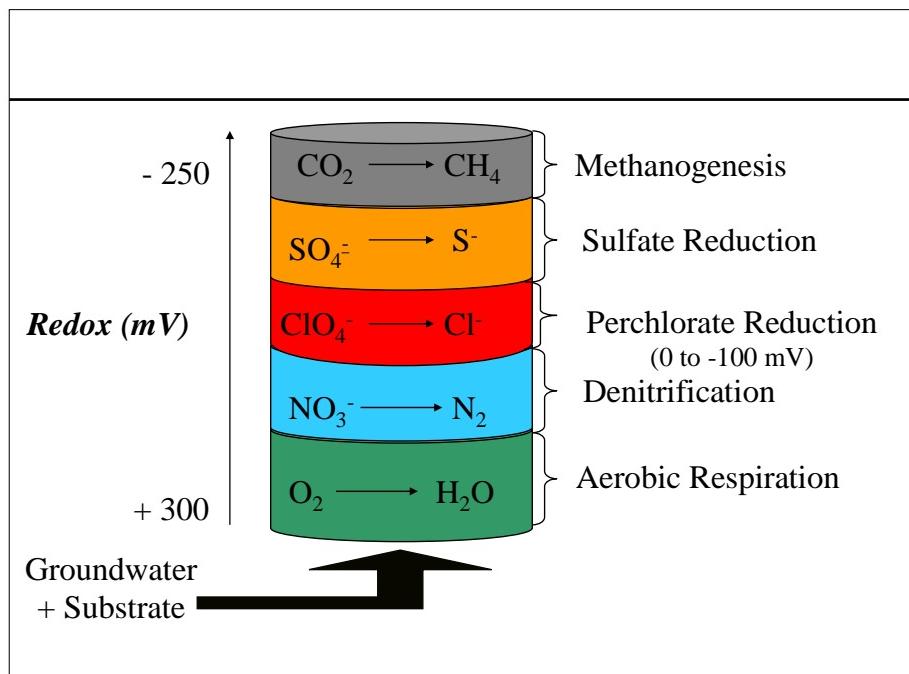


Figure 7. Redox Potential for Degradation Processes.
(ITRC, 2002)

4.2.7 pH and Temperature

The presence and metabolic vitality of microorganism can be affected by pH. For example, dechlorinators are pH sensitive and dechlorination rates decline below a pH of 6. At many sites the pH is naturally low and can inhibit reductive dechlorination. However, the pH issue is further complicated by geochemical changes that occur during anaerobic bioremediation. Reduction of iron and manganese oxides and sulfate will consume H⁺ causing an increase in pH, while CO₂ production and/or fatty acid accumulation during fermentation of complex substrates can cause a decline in pH.

The perchlorate-reducing bacteria generally grow optimally at pH values near neutrality. However, field studies have shown that some species are capable of growth, and perchlorate respiration can occur at pH values as low as 5 (Coates and Achenbach, 2006). In evaluating the potential for MNA of perchlorate, pH values between 6 and 8 are preferable.

As stated by Weidemeier et al. (1995, 1998), “groundwater temperature directly affects the solubility of oxygen and other geochemical species.... Groundwater temperature also affects the metabolic activity of bacteria. Rates of hydrocarbon biodegradation roughly double for every 10°C increase in temperature over the temperature range between 5 and 25°C.” This general rule is expected to apply to species capable of reducing perchlorate in the environment.

4.2.8 Total Organic Carbon and Dissolved Organic Carbon

For perchlorate degradation to occur, an electron donor must be present. TOC concentrations greater than 10 mg/L are preferable for perchlorate degradation, and TOC concentrations greater than 50 mg/L are believed to be more favorable for MNA.

Substrate demand can be described in terms of the electron acceptor demand exerted by the following three categories (ITRC, 2008):

- **Contaminant (Perchlorate) Electron Acceptor Demand.** Since perchlorate serves as an electron acceptor during biological reduction, there is a stoichiometric relationship for the electron donor (e.g., hydrogen) required to meet the electron acceptor requirements.
- **Native Electron Acceptor Supply.** The flux of groundwater and minerals on the solid aquifer matrix include electron acceptors that in many cases are preferentially used over perchlorate. Therefore, their presence exerts a demand on the electron donor required to satisfy the removal of more energetically favorable electron acceptors, which must occur before conditions conducive to anaerobic biological reduction are established.
- **Nonspecific Demand.** One must expect that a large percentage of indigenous organic materials will be used by opportunistic microbes for a myriad of life processes. In addition, numerous transformations of the solid mineral matrix may occur. Thus, there is a nonspecific substrate demand that is not practical to calculate.

The substrate demand must be met until a contaminant source is depleted or until remedial goals have been met. The type of substrate/electron donor can play a role in how thoroughly a natural system is able to transform perchlorate. In some aquifers, the electron donor demand due to the perchlorate flux alone can overwhelm the natural reducing capacity of the subsurface, so MNA may not be a sustainable long-term remedial option (Nzengung et al., 2008). Under MNA conditions, organic carbon may be available as a result of natural processes. For example, root exudates can promote rhizodegradation, making it an effective process in shallow soil contamination situations. Perchlorate degradation half-lives of minutes to hours have been observed, depending on the availability of DOC or other electron donors.

Organic rich sediments found in wetlands, streams, mudflats and riparian buffers also offer potential conditions that would likely support natural attenuation of perchlorate (Borden et al., 2007). Continuous carbon supply may be provided by dead roots and stems, as well as exudates from the root zone (Tan et al., 2004b). However, Hines et al. (2002) examined perchlorate degradation in several depositional environments in the vicinity of missile launch operations including freshwater and marine sediments and peat and observed that perchlorate was not used as a terminal electron acceptor, at least within the one week duration of their evaluation. They did conclude that extended exposure to perchlorate in these environments would likely result in anaerobic biodegradation to occur. In a demonstration project performed at Indian Head,

Maryland, Knox et al. (2007) showed >99 % decrease in perchlorate concentration as groundwater 8 to 10 feet (ft) below ground surface (bgs) contaminated with 10,100 µg/L moved vertically upward through a more organic intertidal mixing zone (See Indian Head, Maryland, case study in the Appendix). Increased TOC (4 mg/L) and methane concentrations (450 µg/L) near the surface were measured, and an increase in the number of prc gene copies correlated with the locations where perchlorate concentrations were reduced. Together these observations formed lines of evidence supporting the conclusions that MNA was occurring.

4.2.9 Trace Metals: Molybdenum

Molecular studies have indicated the presence of a molybdenum-dependent chaperone gene associated with the genes encoding the CD and prc enzymes in certain strains of perchlorate-reducing bacteria (Chaudhuri et al., 2002). In addition, microbial growth and perchlorate reduction were completely inhibited when an active perchlorate-reducing bacterial culture was transferred into medium without molybdenum present (Chaudhuri et al., 2002). The results of these studies suggest molybdenum may be required for perchlorate reduction.

4.3 FIELD METHODS FOR PERCHLORATE ANALYSIS

Field methods for detecting and measuring perchlorate are available. However, their use is not widespread. The ion-selective electrode (Section 4.3.2) and colorimetry (Section 4.3.3) methods are discussed below.

4.3.1 Sample Collection and Preservation

The collection and preservation of perchlorate-contaminated matrices is an important step in obtaining representative and reliable site data. Soil samples can be collected by any number of standard methods such as hand augers or split-spoon samplers and placed in small (4- or 8-oz) glass jars for transport to the laboratory. The presence of air in the soil sample jar is encouraged because it limits biodegradation of the perchlorate during transport. Because perchlorate is water soluble, decontamination of sampling tools should include tap water rinse, soapy wash, tap water rinse and final rinse with deionized water followed by air-drying. Final rinsing of the sampling equipment with isopropanol is not typically needed unless organic solvents are in the soil as co-contaminants. No additional preservation is required for soil samples beyond chilling the soil to approximately 4°C during transport. Further changes or degradation of perchlorate during shipping is limited by lowering the temperature to reduce metabolic activity of the perchlorate-reducing bacteria that may be in the sample.

Aqueous samples can be collected by any number of standard methods including bailers, submersible pumps, and peristaltic pumps, as site conditions allow. Unless new bailers or tubing are being used for each location, submersible pumps or components should be decontaminated with a tap water rinse, soapy wash, tap water rinse, and final rinse with deionized water followed by air-drying. Final rinsing of the sampling equipment with isopropanol is not typically needed unless organic solvents are in the aqueous matrix as co-contaminants. However, because perchlorate is subject to further degradation during transport in aqueous media, additional preservation steps are recommended to improve the stability of the sample.

A method has been developed that takes into account the biodegradability of perchlorate and minimizes potential for degradation of the perchlorate in the groundwater sample during transport and storage prior to analysis. ITRC (2005) describes a procedure that combines field filtration to reduce bacterial content in the sample, followed by packaging with an aerobic headspace to further limit anaerobic biodegradation. The field filtration step should not to be confused with the filtration carried out by the laboratory just prior to ion chromatography (IC) (Method 314 discussed below); the former is for preservation of the sample while the latter is a preparation step for the method. To collect an aqueous perchlorate sample, use traditional collection methods (i.e., bailers, pumps, etc.) to fill a plastic bottle (e.g., 200-mL volume) with groundwater. Allow solids within the sample to settle in the bottle for a brief period of time (approximately 5 to 10 minutes) and then use a 50- or 60-mL plastic disposable syringe to withdraw a sample from the top to avoid solids. Prepare a stack of disposable syringe filters comprised of a single 1.0 μm and 0.45 μm filter (Figure 8). Affix the filter stack to the syringe and push the groundwater through the stacked filters into a clean plastic or glass 40-mL volatile organic analysis (VOA) vial. Fill only 50 to 75% of the vial, leaving the balance of the volume as headspace. Close the bottle while retaining the headspace and place the vial in a cooler on ice for transport.



Figure 8. Photograph Illustrating Field Preservation of the Aqueous Sample By Filtration Through a Filter Stack.

4.3.2 Ion-Selective Electrodes

The Army Corps of Engineers developed an ion-specific electrode for monitoring perchlorate in groundwater monitoring wells (MW), and there is also a commercially available electrode for this purpose (ITRC, 2005). However, the detection limit is approximately 200 $\mu\text{g/L}$ and the electrode is subject to interference by thiocyanate, iodide, nitrate, chloride, phosphate, and acetate, although there is some acceptable level of toleration of chloride and nitrate.

In 2004, the National Defense Center for Environmental Excellence (NDCEE) successfully demonstrated a prototype field instrument to measure down to 10 µg/L perchlorate in water (NDCEE, 2004). The method was a more portable field adaptation of IC in conjunction with an ion selective electrode. At the time, NDCEE was seeking to improve and commercialize the technology, but few reports of perchlorate remediation have reported using this technology for data collection.

4.3.3 Colorimetry

Thorn (2004) described a colorimetric method for perchlorate in water and soil extracts with detection limits reported to 1 µg/L for water and 0.3 µg/g for soil (ITRC, 2005). There are some interferences with the method, particularly in matrices with background coloration such as humic materials and chlorophyll containing materials.

4.3.4 Chemical Sensors

Some efforts have been made to develop dipstick chemical sensors based on molecular recognition events to demonstrate a field, screening level chemical assay for perchlorate ions, and nitroaromatic explosives including TNT and DNT (Strategic Environmental Research and Development Program [SERDP] Project ER-1418). The commercialization and use of this method is unknown.

4.4 LABORATORY METHODS FOR PERCHLORATE ANALYSIS

4.4.1 Analytical Methods

Because of perchlorate's mobility in soil and groundwater and its tendency to form long persistent contaminant plumes, much of the method development work has focused on analysis of perchlorate in aqueous media. Limited methodologies have addressed extraction of perchlorate from soil and other media but, where applicable, these approaches will have importance in the overall identification and quantification process (Canas, 2005; Canas et al., 2006). The following sections discuss the methods available for the determination of perchlorate in aqueous media, principally drinking water. However, as more environmental projects are performed, remediation practitioners are using these methods for non-drinking water determinations. Practitioners are encouraged to consider splitting a portion (e.g., 10%) of samples collected early in the project and running the samples by at least two methods, typically a less selective method and a more selective method. By comparing methods, the user can gain confidence in the analyses and select a preferred method to continue using throughout the project with greater assurance that the results are accurate and representative.

4.4.1.1 Ion Chromatography Methods

In November 1999, the USEPA promulgated an ion chromatographic method for the analysis of perchlorate in drinking water. Since that time several refinements of the basic have been made. The list of IC-based methods for perchlorate that are available includes:

- Method 314.0
- Method 9058
- Method 314.1

In Method 314.0 the perchlorate ion is separated from the introduced aqueous sample and measured using a system consisting of an ion chromatographic pump, sample injection valve, guard column, analytical column, suppressor device, and conductivity detector. The conductivity detector is nonspecific; ions are differentiated based solely on retention times.

Sample matrices with high total dissolved solids (TDS) and high concentrations of common anions such as chloride, sulfate, and carbonate can destabilize the baseline in the retention time window for perchlorate. These can be assessed indirectly by monitoring the conductivity of the matrix. The method requires determining the instrument-specific matrix conductivity threshold (MCT). The method is subject to false positives due to the unspecific nature of the conductivity detector (ITRC, 2005). Some pretreatment by the laboratory to attempt to reduce interferences has the potential to reduce the actual perchlorate content of the sample at low concentrations. The aqueous reporting limit using Method 314.0 is typically 4 µg/L.

Method SW 9058 is the USEPA's Office of Solid Waste (OSW) method for IC determination of perchlorate in aqueous media. The method is substantially the same as Method 314.0, although determining the instrument-specific MCT is not required. The method is stated to perform adequately on water samples with conductivities up to 1,000 µS/cm and is potentially applicable to surface water, mixed domestic water, and industrial wastewaters. The limitations for Method 314.0, including known interferences, false positives, and false negatives, apply similarly to Method SW 9058.

Method 314.1 was adopted in May 2005 by USEPA as an improved IC method for the analysis of perchlorate including samples with high TDS. It is a sample preconcentration, matrix elimination IC method using suppressed conductivity detection for the determination of perchlorate in raw and finished drinking waters. This method is intended to add increased sensitivity, better tolerance of TDS, and better selectivity through use of a confirmation column and in-line concentration. The minimum reporting level (MRL) for the method is below 1 µg/L; the actual MRL depends on sample matrix, fortification concentration and instrument performance. Additional discussion of the applicability and analytical limitations of these IC methods is provided by ITRC (2005).

4.4.1.2 Mass Spectroscopy and Dual Mass Spectroscopy Methods

Coupling mass spectroscopy (MS) to basic ion or liquid chromatography (LC) increases the selectivity and lowers the detection threshold for the perchlorate molecule. A thorough discussion of the most common methods using MS is presented in ITRC (2005). The methods being developed include:

- EPA Method 6850 – Liquid Chromatography/ Mass Spectroscopy (LC/MS)
- EPA Method 331.0 – LC/MS or LC/MS/MS

- EPA Method 332.0 – IC/MS or IC/MS/MS
- Food and Drug Administration (FDA) Method – IC/MS/MS

The LC/MS methods use a liquid chromatograph with a peptide-impregnated reverse phase column to perform the separation followed by a mass spectrometer for detection. Sample pretreatment is not required. This method has been evaluated with drinking water, soil, biota, and synthetic ground water (Di Rienzo et al., 2004). The advantages of LC/MS are the increased sensitivity, increased specificity, the lack of sample pretreatment, and the lack of additional instrumentation. The quantitation limit in water is reported to be 0.1 µg/L.

LC/MS/MS uses a liquid chromatograph with an anion exchange column linked to a triple-stage quadrupole tandem MS with electrospray ionization in the negative mode. The advantage of the LC/MS/MS is the increased sensitivity and specificity based on the parent ions (m/z 99 and 101), the daughter ions (m/z 83 and 85) and the ion ratio of the naturally occurring abundance of ^{35}Cl and ^{37}Cl (3.08). The quantitation limit in water is reported to be 0.02 µg/L.

The IC/MS methods are essentially the same as the IC method; however, a mass spectrometer with an electrospray interface is added. This method requires the use of a suppressor to avoid inorganic salt buildup and uses a conductivity meter to check its efficiency. It uses the mass-to-charge (m/z) 99 and 101 ions for peak identification of perchlorate and monitors the ion ratio of the naturally occurring abundance of ^{35}Cl and ^{37}Cl , which should be 3.08.

The advantages of IC/MS are the increased sensitivity and increased specificity; however, high hydrogen sulfate (HSO_4^-) content will elevate the baseline because it elutes prior to perchlorate. Even with high sulfate concentrations (~1,000 parts per million [ppm]), 0.1 µg/L perchlorate can still be detected. If the baseline is elevated, there is a mandatory cleanup step to remove the sulfate prior to sample injection. The quantitation limit in water is reported to be 0.1 µg/L.

IC/MS/MS method uses an IC coupled with a conductivity detector and a tandem mass spectrometer, thereby increasing the sensitivity and specificity over that of IC/MS. The second MS allows further fragmentation of the perchlorate ions into the daughter ions (m/z) 83 and 85, eliminating false positives or negatives that can be caused by interferences. The quantitation limit in water is reported to be 0.01 µg/L (Penfold, 2004).

4.5 MICROBIAL INDICATORS OF PERCHLORATE ATTENUATION

In many cases, contaminant and geochemical data may be sufficient to demonstrate natural attenuation of perchlorate. The disappearance of the parent perchlorate anion, combined with identification of and eventual disappearance of metabolic degradation intermediates, can be used as supporting lines of evidence for natural attenuation. However, sites with marginal evidence of perchlorate biodegradation may benefit from the use of a variety of microbial screening methods. Microbial enumeration methods can be used to quantify the population density. MBT can be used to characterize the structure, function, and activity of *in situ* microbial communities. Advances in molecular biology have had a profound effect on studies of chlorinated solvent bioremediation processes. Recently, MBTs have been developed to assess the potential for

perchlorate biodegradation. While current use of these MBTs is limited, this technology is evolving very rapidly and there is tremendous potential for these tools to improve. The following sections describe methods for obtaining microbial-mediated data that can be used as lines of evidence for ongoing natural attenuation.

4.5.1 Degradation Daughter Products

The biodegradation pathway for perchlorate was shown in Figure 2. As discussed in Section 3.4.3, perchlorate degradation proceeds through the sequential loss of oxygen from the anion from perchlorate to chlorate (ClO_3^-), chlorite (ClO_2^-), and finally chloride (Cl^-) and oxygen (O_2^-). The rate-limiting step in the three-step degradation process is the conversion of perchlorate to chlorate by a prc enzyme. Subsequent conversion of chlorate to chlorite is also catalyzed by a prc enzyme. Chlorite removal by the CD enzyme is the final step in perchlorate reduction.

Perchlorate can be detected at low concentrations by multiple laboratory methods such as IC, IC/MS, LC/MS, etc. discussed above. Method detection levels can routinely approach 4 $\mu\text{g/L}$ and below, depending on the method used. The disappearance of the parent molecule is presumptive evidence of degradation. The appearance and subsequent disappearance of metabolic breakdown intermediates can further support the claim that biodegradation is occurring. However, intermediates do not ordinarily accumulate in solution during perchlorate biodegradation (Logan, 2001) because the chlorite to chloride step proceeds rapidly, on the order of 1,000 times that of the rate-limiting prc mediated conversion of perchlorate to chlorate (ITRC, 2002). Although chlorate, chlorite, and chloride can all be detected by IC Method 314.0 or 314.1, the detection limit for these anions is typically in the range of 500 $\mu\text{g/L}$, which often precludes the detection of low levels of these intermediates. In the laboratory, stoichiometric conversion of perchlorate to chloride has been observed. However, changes in chloride due to perchlorate biodegradation may be difficult to measure on the field-scale depending on the background concentrations of chloride and the concentrations of perchlorate initially present.

4.5.2 Microbial Enumeration Methods

With the number and variety of microbial species capable of reducing perchlorate, traditional methods such as anaerobic plate counts are used in laboratory studies for enrichment and isolation, but not as frequently in field demonstrations for population enumerations. Coates et al. (1999) utilized laboratory salts media with either chlorate or perchlorate as the only electron acceptor to show that chlorate-reducing bacteria represented a significant population in diverse environments, including pristine and hydrocarbon-contaminated soils, aquatic sediments, paper mill waste sludges and farm animal waste lagoons. Thirteen isolates were collected and shown capable of growth on acetate using perchlorate. The detailed nutritional requirements of the perchlorate reducing bacteria remains largely unknown, and artificial media incorporating various buffers, vitamin solutions and trace mineral mixtures had all been tried (Xu et al., 2003).

Wu et al. (2001) developed a most probable number (MPN) method with anaerobic growth medium to enumerate perchlorate-reducing bacteria and chlorate-reducing bacteria. They concluded from their study of natural waters, soil, and wastewater that bacteria capable of chlorate reduction appear to be more abundant than those able to degrade perchlorate. Kesterson

et al. (2005) used MPN incubated under anoxic conditions and reported from <20 to 230 perchlorate-reducing bacteria/100 milliliters (ml) to <20 to $>1.5 \times 10^5$ /g for Lake Mead water and Las Vegas Wash sediments, respectively. Choi and Silverstein (2008) used MPN to estimate perchlorate- and nitrate-reducing populations in bioreactor biofilms. Logan et al. (2001), Rikken et al. (1996), and others have used selective growth media to isolate bacteria capable of growth on perchlorate. Although some studies can be found that use these methods, relatively few remediations use these traditional methods to track changes in microbial populations of the perchlorate-reducing bacteria as part of the demonstration of effectiveness of the remedial strategy.

4.5.3 Perchlorate Reductase and Chlorite Dismutase Enzyme Assays

Newer, more specific real-time PCR methods are commercially available that can provide a sensitive, rapid approach to qualitatively detect (i.e., the PCR assay) or quantify (i.e., the qPCR assay) specific microorganisms involved with bioremediation. These methods can be applied selectively to detect and/or enumerate the proportion of active perchlorate-reducing bacteria in a total population of bacteria by targeting specific genes found in these organisms.

The MBTs that have been developed (and are under continuing refinement) for the perchlorate-reducing bacteria are based on the specificity of the prc and CD enzymes. Perchlorate reductase is the enzyme that mediates the initial breakdown of perchlorate to chlorate and chlorite; it is coded for by the prc gene operon which consists of subunits *pcrA* through D. CD is the single enzyme that mediates dismutation of chlorite, the final step in reduction of perchlorate to chloride and oxygen; it is coded for by the *cld* gene (Gunawan, 2007). These genes and enzymes are common to all perchlorate-reducing bacteria.

In general, MBTs for perchlorate-reducing bacteria are based on the same general method applied to the different genetic material: DNA-based and RNA-based PCR. DNA-based approaches are used to determine if bacteria with the genetic potential to degrade perchlorate are present and, if so, at what concentration. This approach simply tells the user whether or not capable bacteria are present; however, it is not a measure of induced, stimulated, or naturally occurring bioactivity. By contrast, RNA-based measurements are used to determine that the perchlorate-reducing bacteria are actively producing the enzymes, presumably for metabolizing perchlorate (Achenbach et al., 2006).

At this time, Microbial Insights, Inc. (Rockford, Tennessee) offers DNA-based and RNA-based PCR assays for both genes. Either assay may be performed qualitatively or quantitatively. The DNA-based assay determines the presence of the functional *pcr* or *cld* genes in the sample matrix. Qualitative results from this approach determine if organisms with the genetic potential to degrade perchlorate are present or absent in the sample provided. If quantitation is desired, the method reports the number of gene copies per unit volume. However, the presence of the gene copies alone does not indicate that the bacteria are alive and metabolically active or expressing a particular function, i.e., whether or not perchlorate-reducing activity is actually occurring. Borden et al. (2007) reported the results of qualitative DNA-based CD assays on site

matrices from seven DoD sites. The CD assay was useful in determining the potential presence of organisms capable of reducing perchlorate in the various environments included in the study.

RNA-based PCR measurements are a more direct indicator of enzymatic activity. They can also be applied to detection of either the *prc* or *CD* genes. However, in this method, RNA is extracted from the microbial population in the sample. The RNA is then analyzed using the PCR assay for the detection of the desired gene (i.e., *prc* or *cld*). The RNA is used to determine the expression of the particular functional gene, based on the abundance of messenger RNA (*mRNA*). The perchlorate-reducing bacteria use the *mRNA* to assemble the enzyme, and the abundance of *mRNA* in the groundwater sample is an indirect indication of enzyme production and, therefore, active biodegradation of perchlorate.

Because of the simplicity, growing availability and accuracy of these methods, their use in monitoring the effectiveness of remediation will likely increase. At this time, the DNA-based qPCR assays are more stable and less subject to sample collection and matrix variability. The RNA is more difficult to perform and generally results in lower concentrations reported (personal communication, Microbial Insights, August 2008). Clearly however, evidence of even few RNA-based gene copies is direct evidence of perchlorate metabolism, whereas DNA-based results should be considered along with all other lines of evidence.

4.5.4 Stable Isotope Methods

One difficulty in demonstrating natural attenuation is distinguishing perchlorate removal due to biodegradation from other loss mechanisms, such as dilution, dispersion, and chemical reactivity etc. Stable isotope analysis provides a method for distinguishing biotic from abiotic attenuation since microbial processes are known to make small but significant changes to isotopic compositions of many molecules (Coleman et al., 2003).

Microorganisms often preferentially use lighter isotopes in their metabolic processes (Mariotti et al. 1981; Heaton, 1986), and, as a contaminant is degraded, the isotopic composition of the remaining material becomes progressively heavier. This isotopic shift can be described by the Rayleigh Distillation formula, $R/R_0 = f^{(\alpha-1)}$, where R_0 is the isotopic ratio of the original material, R is the isotopic ratio of the remaining material, α is the fractionation factor and f is the fraction of material degraded. If the ratio R/R_0 can be accurately measured and α is known, the fraction of material degraded can be calculated.

A variety of different investigators have successfully used stable isotope ratios to evaluate the MNA of petroleum hydrocarbons (Ahad et al., 2000), MTBE (Kolhatkar et al., 2002), chlorinated solvents (Lollar et al., 2001), and nitrate (Karr et al., 2001). However, there are some important limitations to this approach: 1) a sensitive, reproducible method is needed to monitor the isotopic shifts; 2) variations in the isotopic composition of the different sources can mask isotopic shifts caused by microbial fractionation; and 3) the isotopic fractionation factor α may vary between different microorganisms and environmental conditions (Slater et al., 2001).

Monitoring isotopic ratios may be a very useful tool for evaluating the extent of perchlorate attenuation. The isotopic signature of both the oxygen atoms ($^{18}\text{O}/^{17}\text{O}/^{16}\text{O}$) and chloride atoms ($^{37}\text{Cl}/^{35}\text{Cl}$) on perchlorate are useful for distinguishing sources of perchlorate as well as evidence of biodegradation-mediated changes (Sturchio et al., 2006; Bohlke et al., 2005). Bohlke et al. (2005) indicated that there was some evidence that microbial reduction of perchlorate caused the $\delta^{18}\text{O}$ to increase about two times as fast as $\delta^{37}\text{Cl}$.

More studies have examined the isotopic shifts of the chlorine atoms in perchlorate. Ader et al. (2001) developed a highly reproducible and accurate method for stable isotopic analysis of chlorine ratios $^{37}\text{Cl}/^{35}\text{Cl}$ ($\delta^{37}\text{Cl}$) in perchlorate. Coleman et al. (2003) observed that perchlorate reduction by *D. suillum* resulted in significant fractionation (~ -15‰) of the chlorine stable isotopic composition. The resulting shifts in $\delta^{37}\text{Cl}$ associated with perchlorate reduction were much larger than the isotopic variations between different sources (+0.2‰ to +2.3‰) observed by Ader et al. (2001). These results suggest that isotopic ratios could be used to assess biodegradation of perchlorate in the field and separate biodegradation from nonbiological loss mechanisms.

To use stable isotope methods to evaluate perchlorate attenuation at a site, groundwater samples should be collected and assayed for $\delta^{37}\text{Cl}$ or $\delta^{18}\text{O}$ of perchlorate. The sampling locations should include locations where groundwater conditions suggest that perchlorate may be biodegrading as well as background locations. Depending on the concentrations of perchlorate present in the groundwater, large volumes of groundwater may be needed to perform the stable isotope analysis. Typically 10 mg of perchlorate are necessary for the analysis. For sites with low perchlorate concentration, a small portable ion exchange resin is used to trap the necessary sample mass as the groundwater is collected. Groundwater is pumped through columns containing the resin at a low flow rate until the column contains approximately 10 mg of perchlorate (Bohlke et al., 2005). Each cartridge is then shipped to a laboratory for perchlorate extraction and isotope analysis. The data are used to assess spatial variations in $\delta^{37}\text{Cl}$ or $\delta^{18}\text{O}$ to determine if there is significant isotopic fractionation during downgradient transport. If there is clear evidence of isotopic shifts, the extent of perchlorate degradation can be estimated using the fractionation factor. Hatzinger et al. (2007) demonstrated the isotopic shift in a permeable reactive barrier created by the injection of an Emulsified (edible) Oil Substrate (EOS®) perpendicular to a perchlorate groundwater plume. Perchlorate-contaminated groundwater was extracted from an upgradient well, pumped into the barrier, extracted over time, and analyzed. Perchlorate levels declined from 4300 µg/L to 500 µg/L during an 8-hr period, and the corresponding data measured significant fractionation of both isotopes: $\delta^{37}\text{Cl}$ (-2.9‰) and $\delta^{18}\text{O}$ (-7.9‰). These changes were clear indications that biodegradation of the perchlorate was being stimulated in the permeable reactive barrier.

4.6 LABORATORY MICROCOSMS AND BENCH-SCALE COLUMN TESTS

Laboratory microcosms and bench-scale column studies can be used to demonstrate that natural attenuation is occurring and to estimate the rate and extent of perchlorate biodegradation. Microcosms and bench-scale column studies can be time-consuming and expensive and should not be employed until a considerable understanding of site conditions has been achieved through

site investigation activities. The studies should be conducted using matrices that are representative of the prevailing geochemical conditions at the site. Careful planning should also be used in developing a sampling strategy and determining the duration of the study. These factors can greatly influence the results of the studies.

4.6.1 Laboratory Microcosm Studies

Microcosm studies typically consist of collecting representative aquifer material from the site and using this material to construct microcosms with different experimental treatments. At a minimum, treatments should include ambient site conditions and a killed control. Depending on the perchlorate levels found at the site, it might also be desirable to evaluate a treatment with a spiked, higher concentration of perchlorate. Whenever possible, for statistical purposes, each treatment should be run in triplicate. Typical sampling might include perchlorate, chloride, chlorite, chloride, DO, ORP, pH, TOC, and nitrate.

Previous studies have compared laboratory microcosm rates with field attenuation rates at multiple sites (Borden et al. 1995, 1997a, 1997b; Johnston et al. 1996; Hunt et al. 1998). This experience indicates that laboratory microcosms sometimes provide a very good prediction of field degradation rates. However, at other times, the microcosm rates do not match field rates. When microcosm and field rates do not match, the differences appear to be because (a) levels of electron acceptor or donor in the microcosms do not match field conditions and (b) the microbial distribution in the aquifer is very heterogeneous and blending of the aquifer material for the microcosm study enhances contact between the microbes, electron acceptors, and electron donors. As a consequence, great care must be taken when extrapolating laboratory rates directly to the field. However, whenever contaminant biodegradation is observed in the laboratory microcosms, field biodegradation is usually also observed. Conversely, when contaminants do not degrade in the laboratory, they do not usually degrade in the field. As a consequence, laboratory microcosms can provide a very useful indication of the potential for natural attenuation. However, laboratory biodegradation rates should not be used to estimate field rates without accounting for differences in environmental conditions.

Microcosm studies assessing the natural attenuation of perchlorate have been performed previously, albeit infrequently. In many of these studies, the emphasis has been on the existence and enhancement of native microbial populations under various conditions, with little interest shown to the results of the “ambient control,” which reflects the potential for natural attenuation in the particular environment under study. For example, Borden et al. (2006) demonstrated the relatively quick biodegradation of 53,000 µg/L perchlorate to less than 8 µg/L in laboratory microcosms in less than 14 days when provided with excess electron donor. The ambient control did not change in the same period; because it was not the focus of the study, natural attenuation was not monitored any further.

Wu et al. (2001) studied persistence of perchlorate in waters, soil, and wastewater while evaluating the population density of perchlorate-reducing bacteria in these environments. Their findings supported the assertion that perchlorate- and chloride-respiring bacteria were widely distributed in nature, and the initial population density likely reflected prior exposure to

perchlorate contamination. Thus, intrinsic perchlorate remediation was more likely limited by a lack of suitable environmental conditions.

Hines et al. (2002) examined the impact of up to 1,000 mg/L perchlorate on microbial respiration in freshwater and marine sediments and in bog peat. Their studies showed generally no inhibition of CO₂ production or methanogenesis as a result of increasing perchlorate concentrations. Their incubations lasted only one week and also showed no measurable degradation of the added perchlorate by indigenous microbes during this time. This was attributed to lack of constitutive ability to utilize perchlorate by native microorganisms that had not previously been exposed to perchlorate.

Jackson et al. (2002) calculated a pseudo-first-order degradation rate of 0.185 day⁻¹ in an unamended control prepared from contaminated soil containing 10 mg/kg perchlorate. Tan et al. (2004a) performed a series of microcosm tests on perchlorate-contaminated sediments from Naval Weapons Industrial Reserve Plant (NWIRP) in McGregor, Texas, and soil from Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas, to investigate the potential of intrinsic perchlorate biodegradation in these matrices. Background organic substrate availability and the presence of nitrate were found to be crucial factors affecting microbial degradation rates and lag times. Intrinsic perchlorate degradation rates ranged from 0.13 to 0.46 day⁻¹ corresponding to a half-life range of 1.4 to 5.0 days. The concentration of nitrate affected lag time, but not degradation rate, and in contrast to earlier findings, pre-exposure to perchlorate did not affect the biodegradation rate.

In a recent-ESTCP-funded evaluation, Borden et al. (2007) screened multiple DoD-related perchlorate-impacted sites located throughout the United States to identify sites where natural attenuation processes may be important. Groundwater and soil/sediment samples were collected from seven sites, characterized, and used in microcosms to evaluate the potential for natural attenuation of perchlorate to occur. Perchlorate concentration trends, biological activity indicators, electron acceptor and oxidation-reduction conditions, and organic carbon status were evaluated. CD enzyme assays suggested that genetically capable microbes were ubiquitous, but the rates of degradation varied widely. Degradation rates were calculated for each site and fitted to one of two kinetic models. The degradation rates were zero-order at two of the seven sites (Alliance Techsystems [ATK] Elkton and ATK Thiokol), 1st order at two sites (Naval Surface Warfare Center [NSWC] Indian Head and Redstone Arsenal) and could be fitted to both models at one site (Stennis Space Center). At two other sites (Little Mountain Texas Annex [LMTA] and Beale Air Force Base [AFB]), apparent zero-order biodegradation rates were less than 0.01 mg/L per year, indicating no measurable perchlorate degradation. When measurable, zero order rates ranged from 0.13 mg/L/yr at Stennis Space Center to 33 mg/L/yr at ATK Thiokol. The first order rates ranged from 1.75 yr⁻¹ at Stennis Space Center to 5.1 yr⁻¹ at NSWC Indian Head.

All sites showed low nitrate (<10 mg/L); sites with lower redox potentials showed greater potential for natural attenuation. TOC in groundwater was less than 4.4 mg/L at all the sites, but there was sufficient carbon to support some degree of attenuation. Sites with neutral pH, low redox potential, low nitrate and elevated TOC would be expected to support perchlorate-degrading bioactivity.

4.6.2 Bench-Scale Column Studies

Bench-scale column studies can be used to evaluate biodegradation rates in simulated natural environments. Tipton et al. (2003) used 15-cm long by 7.5-cm i.d. columns to evaluate bromide and perchlorate transport through two California loam soils. The results demonstrated that biodegradation has the potential to affect the transport of perchlorate in native soils (unamended with nutrients or carbon) and that more biodegradation occurred in soils previously exposed to perchlorate. Further, lower hydraulic conductivity led to increased contact time yielding more biodegradation.

Tan et al. (2004b) examined the potential of natural wetland cores to treat perchlorate-contaminated water in vertical upflow wetland columns with and without native bullrushes. Intact cores were collected from a freshwater wetland in Madisonville, Louisiana. In unplanted columns without nitrate, up to 32 mg/L perchlorate were removed effectively by the core sediments, but the rate of perchlorate biodegradation decreased from 6.49 to 0.42 day⁻¹ as the concentration of perchlorate and nitrate increased. Degradation was complete after a 9.6-day residence time in the 55-cm column. In planted columns, a mass balance indicated plant uptake accounted for transformation of 0 to 14.3% of initial perchlorate input. Microbial degradation played a more important role than plant uptake and transformation in the simulated wetland system.

4.7 FIELD EVALUATION TOOLS AND TECHNIQUES

4.7.1 In Situ Columns

The in situ column method was used by Borden et al. (1997a) to evaluate anaerobic biodegradation of benzene. Figure 9 shows the observed loss of benzene in the anaerobic in situ column experiments. The apparent first order decay rate can be calculated as the slope of the live columns minus the abiotic column. Using this procedure, Borden et al. (1997a) showed that decay rates measured in the in situ columns provided a better match with plume-scale degradation rates than conventional laboratory microcosms.

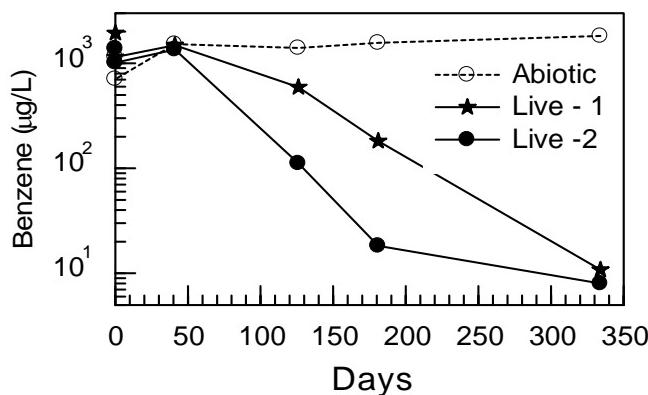


Figure 9. Anaerobic Benzene Loss in In Situ Columns.

In situ columns can be installed at a location where there is evidence that perchlorate is degrading. During the in situ biodegradation studies, columns of soil and groundwater, representative of the aquifer of concern, are isolated from the rest of the formation with a solid structure such a polyvinyl chloride (PVC) casing or pipe. The soil and groundwater within the columns are then treated with perchlorate so that a known concentration of perchlorate is present to start, and potentially the rate of perchlorate biodegradation can be monitored over time within a controlled environment. Biodegradation rates obtained from the in situ biodegradation study may provide another line of evidence with which to evaluate perchlorate MNA.

Close-ended columns can be constructed in a manner that is similar to that reported by Radtke and Blackwelder (2005). The column design is also similar to that used by Gillham et al. (1990), Borden et al. (1997a), and Sandrin et al. (2004). Each column typically consists of a 1-m long chamber that is pushed into the sediment surface allowing sediment and groundwater to be isolated from the surrounding aquifer for controlled observation. To use, groundwater is extracted from the column (or an adjoining well), amended with a nonreactive bromide tracer and perchlorate (if required), and injected back into the column. In the upflow mode, groundwater samples are collected from each column immediately after injection and then on a prescribed performance monitoring schedule and analyzed for perchlorate, chlorate, chlorite, chloride, and bromide tracer, if used. Other parameters that could be monitored include DO, ORP, TOC, and nitrate. By comparing perchlorate concentrations over time, in situ biodegradation rates can be estimated. The nonreactive tracer (Br^-) can be used to evaluate possible dilution effects that might occur during the incubation period. This in situ measurement approach is expected to be most appropriate when groundwater flow rates are low.

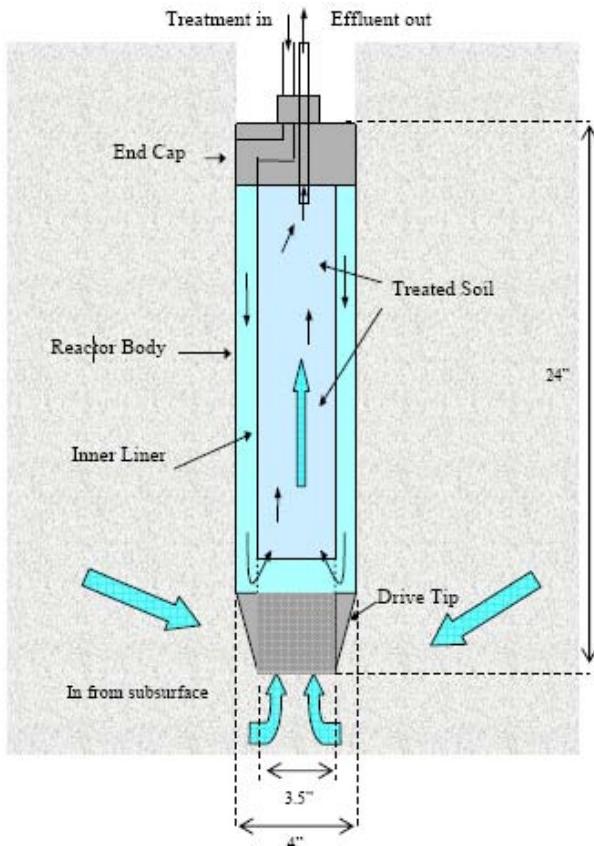


Figure 10. In Situ Column Set-Up.
(Radke and Blackwelder, 2005)

The case study at the ATK Elkton, Maryland, project site provided in the Appendix illustrates the design and application of in situ columns for determining perchlorate degradation rates in the field. In situ columns were installed adjacent to groundwater MWs with known concentrations of perchlorate. The wells were located toward the toe of the perchlorate plume near a stream considered to be a groundwater discharge feature. Three columns were installed below the groundwater table with tubing through the vadose zone to provide amendments and sampling access. Contaminated groundwater was mixed with bromide tracer and pumped into each column; one column was poisoned with nitric acid as a control. Perchlorate concentrations in replicate live columns decreased by 33% from 99.3 to 65.0 µg/L in 43 days (0.80 µg/L/day) while only 10% from 101 to 109 µg/L in the killed control (0.27 µg/L/day). Thus, monitoring changes in perchlorate concentrations in the columns further supported natural attenuation in this area of the plume.

4.7.2 Plume Stability, Statistical Evaluations, and Mass Flux

Historical site groundwater data for perchlorate should demonstrate a clear trend of decreasing concentration and/or mass over time. There are several ways to evaluate historical perchlorate data to show changes in concentrations over time. The simplest method includes preparing

isopleth maps of perchlorate over time and generating graphical plots of perchlorate versus time. Perchlorate is not sorbed to any large degree and therefore is expected to migrate at a rate that is close to the average groundwater flow velocity. By comparing the overall plume size to the expected plume size, given an estimate of when perchlorate first impacted groundwater, one can evaluate whether the plume is stable. If the plume is somewhat shorter than expected, one might conclude that natural attenuation processes are at work.

Statistical techniques can also be used to evaluate plume stability. These techniques include regression analyses, the Mann-Whitney U Test, the Mann-Kendall Test, and center of mass calculations, as described in *Principles and Practices of Enhanced Bioremediation of Chlorinated Solvents* (AFCEE, 2004).

Reduction in perchlorate concentrations alone does not prove that the perchlorate is being biodegraded. The observed reductions could be due to nonbiological process such as dispersion and dilution. However, changes in molar concentrations of perchlorate, chlorate, chlorite, and chloride can be used to evaluate biodegradation of perchlorate. Conventional concentration data can be converted into molar concentrations using the molecular weight of each compound (Table 2). The molar ratio of perchlorate to degradation products can be calculated over time and across the plume. Change in the molar ratio of perchlorate to degradation products can demonstrate perchlorate biodegradation. However, due to difficulties measuring perchlorate degradation products, this method may have limited usefulness on the field-scale.

While the classical methods of evaluating plume dynamics have been discussed briefly above, one additional line of evidence is increasingly being considered by those conducting or overseeing groundwater remediation efforts. This approach compares mass flux along the plume and can be used to produce estimates of the contaminant source strength, i.e. the total mass discharge rate to groundwater and surface water (Annable et al., 2005). By using mass fluxes instead of point concentrations, one can minimize the effects of vertical and transverse dispersion and suboptimal well placement (Borden et al., 1997b). One method to evaluate changes in the mass flux of perchlorate is to install monitor wells as transects across the contaminant plume. The mass flux across one transect can then be compared to downgradient transects to monitor the change in the downgradient mass flux of perchlorate (O'Toole et al., 2004). Adequate evaluation of mass flux changes using this method may require the installation of multiple monitor wells screened at varying depths with numerous samples collected over multiple years. The horizontal spacing of the wells should be sufficient to encompass the entire width of the perchlorate plume. To reduce costs, existing monitor wells and data can be used to the maximum extent possible. If done properly, the information gained can be used in groundwater models to help manage contaminated sites. When measured at locations downgradient from the source, mass flux measurements can be used to verify remediation technology performance, assess natural attenuation rates and evaluate environmental risks (Annable et al., 2005).

These approaches can suggest that perchlorate is naturally attenuating. When properly applied, they offer the first line of evidence that MNA may be influencing contaminant fate at the site. However, for MNA to be accepted, these tests should be included with the other evidence discussed in earlier sections of this document.

5.0 ASSESSING THE NATURAL ATTENUATION OF PERCHLORATE: A TIERED APPROACH

5.1 PURPOSE OF THIS SECTION

The evaluation of the potential for using MNA as a groundwater remedy for all contaminants requires consideration of multiple lines of evidence. In the case of perchlorate, biodegradation is especially important for MNA because perchlorate is not readily sorbed, volatilized, or abiotically degraded (Nzengung et al., 2008). Analytical methods are available to monitor the concentration of perchlorate in the environment with high sensitivity and selectivity, and newer biological tools are under development to identify perchlorate-reducing bacterial population sustainability and activity. However, other than the disappearance of the parent anion, the demonstration of perchlorate degradation has often relied on circumstantial evidence involving the influence of biogeochemical parameters such as pH, DO, redox potential, TOC, DOC, nitrate, sulfate and trace minerals.

As stated in Nzengung et al. (2008), “The biodegradation pathways are well understood and the microorganisms involved in perchlorate biodegradation are known, they can use a variety of different organic substrates as electron donors, are relatively ubiquitous in soil and groundwater environments, and function as strict or facultative anaerobes. This suggests that natural attenuation of perchlorate should occur at many sites (Cooley et al., 2005), and that MNA can be effective in managing the risks posed by perchlorate contamination of groundwater under favorable conditions.” The frequency of sites with suitable conditions that would be expected to sustain natural attenuation of perchlorate is not easily quantified. To date, many site-specific evaluations have focused solely on changes to perchlorate concentrations over time and distance but have not provided sufficient supporting evidence to justify regulatory acceptance of MNA. Therefore, more in-depth, site-specific evaluations, including greater understanding of the conditions that influence perchlorate behavior and degradation, will be important to the application of MNA. Lines of evidence for MNA typically rely on three layers of testing (USEPA, 1999):

- Tier 1—Spatial and temporal distribution of perchlorate
- Tier 2—Bio-geochemical conditions for perchlorate biodegradation
- Tier 3—Microbiological indicators of perchlorate biodegradation.

This section shows how the processes, tools, and monitoring techniques discussed in detail in earlier sections of this document can be applied in each tier specifically for the evaluation of the MNA of perchlorate. This section will summarize the findings and provide a framework for practitioners to systematically approach the problem.

5.2 TIER 1—SPATIAL AND TEMPORAL DISTRIBUTION OF PERCHLORATE

Plume Stability and Geometry. Historical data can be used effectively to delineate the extent of the contamination and determine the fate of contaminants of concern (and any toxic by-products). With a properly designed monitor well network, trends in the data can successfully illustrate plume geometry and stability. Ideally, to support acceptance of MNA, one should show that the contaminant plume is stable or retreating. A stable or shrinking perchlorate plume

would indicate that biodegradation is removing perchlorate from the groundwater at least as fast as the source is releasing it to the plume.

As discussed in Section 4.7.2, historical site groundwater data for perchlorate should demonstrate a clear trend of decreasing concentration and/or mass over time. The simplest tools that are available include visual isopleth maps and concentration trend analysis versus time. Relatively simple statistical techniques can also be used to evaluate plume stability. These techniques include regression analysis, the Mann-Whitney U Test, the Mann-Kendall Test, and center of mass calculations. A more recent and often more costly approach to evaluating contaminant changes is measuring and modeling mass flux across the plume. The practitioner is cautioned when using these methods to seek understanding and approval by the regulatory community before investing extra time and money in applying these to a particular site.

The challenge at all sites is to understand the inherent temporal and spatial variabilities so the data obtained are meaningful and can be applied correctly to the selected evaluation. This may require extensive monitoring and data acquisition over long periods of time before the chosen method can be used effectively and the results relied upon with confidence. These tools comprise Tier 1 and can offer the first line of evidence that perchlorate is naturally attenuating, or at a minimum, not changing. However, this alone is unlikely to be sufficient to make the case for MNA.

5.3 TIER 2—BIOGEOCHEMICAL CONDITIONS FOR PERCHLORATE BIODEGRADATION

Next to looking at trends in the data to demonstrate contaminant concentration or mass changes over time and distance, the collection of site-specific biogeochemical information is the best understood and most widely employed step to provide evidence supporting the potential for MNA of contaminants. The data are evaluated for their affect on targeted biological activity on the contaminant of concern. For petroleum hydrocarbons, which are generally biodegradable under aerobic conditions, this typically includes the evaluation of DO, ORP, pH, and possibly nutrient status (i.e., NH₃-N and PO₄-P, which are needed for microbial growth). For chlorinated solvents, a list of more than 20 parameters has been proposed as a scoresheet (Wiedemeier et al., 1998) that can be used to predict the potential for MNA of many chlorinated ethene and ethane solvents. Many of the same parameters important for natural attenuation of chlorinated solvents are equally important for assessing the potential for perchlorate to biodegrade. The practitioner must keep in mind that these groundwater parameters are not direct measures of ongoing degradation but serve as indicators that identify whether favorable conditions for natural attenuation of perchlorate are present.

The individual biogeochemical factors that can influence the biodegradation of perchlorate were discussed in Section 4.2 of this document. The optimal conditions for the process to occur are summarized below:

- **Dissolved Oxygen and Oxidation-Reduction Potential.** No or low DO and negative redox potentials are necessary for optimal biodegradation. Some perchlorate-reducing bacteria are facultative anaerobes and can tolerate low

oxygen tension, but in general, anaerobic processes are favored. The ORP in the range of 0 to -100 mV provides a favorable reducing environment.

- **Total (or Dissolved) Organic Carbon and Methane.** The presence of available organic carbon to serve as an electron donor (e.g., reduced organic compounds) is another key condition necessary for perchlorate biodegradation. TOC concentrations greater than 2 mg/L are preferable for perchlorate degradation, and TOC concentrations greater than 10 mg/L are believed to be more favorable for MNA. Naturally occurring sources of carbon can be found in wetlands, mudflats, and riparian buffers. Where perchlorate plumes discharge into these features, the opportunity for MNA increases; in mineral soils with little TOC, this may be limited.

Methane can occur in groundwater as a result of biodegradation of organic matter. The source of the TOC may be naturally occurring or anthropogenic (e.g., a co-mingled fuel spill). Methane is not an indicator of the biodegradation of perchlorate but can be used to support the conclusion that the aquifer is strongly reducing, a condition that favors perchlorate reduction.

- **Nitrate and Sulfate.** The impact of nitrate concentrations on potential for perchlorate biodegradation is not absolute, and there is no one concentration of nitrate below which perchlorate reduction is considered optimal. In general, the same conditions that are required for denitrification (the conversion of nitrate to nitrogen gas) are favorable for perchlorate reduction. Many perchlorate-reducing bacteria can reduce nitrate as well as perchlorate (Herman and Frankenberger, 1998), and denitrifying bacteria have been shown to reduce perchlorate (Logan et al., 2001). Nzengung et al. (2008) suggested that a decrease in nitrate coupled with nitrite production, along with a decrease in perchlorate concentration along the flow path, may be good indicators of the natural attenuation of perchlorate. However, if the concentrations of nitrate and other electron acceptors such as oxygen are too high, they can inhibit perchlorate reduction (Chaudhuri et al., 2002; Krauter et al., 2005). This may be a problem in some situations because nitrate levels in groundwater can be orders of magnitude higher than the perchlorate levels. Ideally, low nitrate concentrations provide less competition with perchlorate as an electron acceptor in the environment. However, in presence of excess organic carbon, this may not be as important.

Sulfate is another electron acceptor often found in aqueous environments. Sulfate concentrations have not been shown to have a measurable impact on perchlorate reduction activity. Figure 7 in Section 4.2.6 illustrates that anaerobic perchlorate reduction can occur at redox potentials higher than those that are required for sulfate reduction. Therefore, the presence of sulfate is not a major detriment to perchlorate reduction.

- **Temperature and pH.** The presence and metabolic vitality of microorganisms can be affected by pH and temperature. The perchlorate-reducing bacteria

generally grow optimally at pH values near neutrality. However, field studies have shown that some species are capable of growth and perchlorate respiration can occur at pH values as low as 5 (Coates and Achenbach, 2006). In evaluating the potential for MNA of perchlorate, pH values between 5 and 8 are preferable. Warmer temperatures promote increased activity.

- **Chloride.** If starting concentrations of chloride are low and perchlorate is high, increased levels of chloride may also be directly indicative of perchlorate biodegradation. However, in many situations the chloride background is often relatively high and changes that could be attributable to perchlorate biodegradation are negligible. Perchlorate reducers can also be extremely salt-tolerant (Logan et al., 2001), but those considering the use of chloride as an indicator must be careful to separate naturally occurring concentrations from any additions as a result of biodegradation.
- **Iron.** An increase in dissolved iron Fe(II) can be an indicator of a reducing environment that is conducive to perchlorate degradation. When dissolved iron concentrations are greater than 0.5 mg/L, this indicates anaerobic conditions with a high potential for perchlorate biodegradation.

A consistent and explainable relationship between lines of evidence obtained in Tier 1 and Tier 2 testing may be sufficient to propose MNA as a viable groundwater remedy. If the groundwater conditions are optimal and the plume is stable or shrinking, the conclusion may be easily supported. Unfortunately, most sites are not as clear cut. In these situations, additional evidence may be needed.

5.4 TIER 3—MICROBIOLOGICAL INDICATORS OF PERCHLORATE BIODEGRADATION

In situations where additional lines of evidence are required, Tier 3 testing can be employed. Tier 3 offers several methods that provide direct evidence of biodegradation of perchlorate. The number of tests that are used and the sequence of the testing are not critical. Both laboratory and field tests are available and can be performed. These tests can be useful in determining rates of perchlorate degradation which can be factored into the overall acceptance of MNA as the groundwater remedy.

5.4.1 Perchlorate Reductase and CD Enzyme Analysis

Specific real-time qPCR methods that are commercially available can provide a sensitive, rapid approach to detect and quantify specific microorganisms involved with bioremediation. The methods can enumerate the perchlorate-reducing bacteria in a total population of bacteria by quantifying the *pcr* or *cld* gene copies found in the site matrix. DNA-based qPCR assays provide evidence that perchlorate-reducing capability is present in the environment. Used in conjunction with findings from Tier 1 and 2, this can be considered an important line of evidence for natural attenuation of perchlorate.

The RNA-based qPCR assays for the *pcr* and *cld* genes provide a direct indication of ongoing perchlorate bioremediation activity. Where possible, this is a preferred analysis, but the test is not as well developed as the DNA-based qPCR assay. RNA-based assays have the potential to stand alone as definitive evidence that bioremediation is occurring. However, at this time, results of this assay should also be considered in conjunction with additional lines of evidence from the preceding Tiers.

5.4.2 Laboratory Evaluations

Microcosms and bench-scale column studies can be time-consuming and expensive and should not be employed until a considerable understanding of site conditions has been achieved through site investigation activities (Tier 1 and Tier 2). These studies can be used to demonstrate that natural attenuation is occurring and to estimate the rate and extent of perchlorate biodegradation. Under favorable biogeochemical conditions, including the presence of excess electron donor, the biodegradation of perchlorate has been shown to be relatively quick and can substantially reduce the time needed to acquire data. In laboratory microcosms, Borden et al. (2006) showed a decrease from 53,000 µg/L to less than 8 µg/L in laboratory microcosms amended with an emulsified oil substrate in less than 14 days. However, evaluations of natural attenuation take longer.

The studies must be conducted using matrices that are representative of the prevailing geochemical conditions at the site. Careful planning should also be used in developing a sampling strategy and determining the duration of the study. These factors can greatly influence the results of the studies. The setup of typical microcosm studies was described in Section 4.6.1 along with advantages and precautions associated with the approach.

Laboratory microcosms can provide a very useful indication of the potential for natural attenuation. However, laboratory biodegradation rates should be used carefully to estimate field rates. Differences in actual field conditions compared with the microcosm setup can lead to non-representative results. In a series of microcosm studies conducted as part of this ESTCP-funded project, microcosm studies were prepared on matrices from seven perchlorate-impacted sites located throughout the United States (Borden et al., 2007). Degradation rates were calculated for each site and fitted to zero-order and/or first order kinetic models resulting in a wide range of rates. The microcosm results supported the Tier 1 and Tier 2 findings that conditions were favorable for natural attenuation at several of the sites. Biodegradation was observed in matrices from most sites without the addition of organic carbon (electron donor) or nutrients.

Bench-scale column studies can also be used to evaluate biodegradation rates in simulated natural environments. Using intact cores of sediment, perchlorate degradation can be evaluated in a controlled environment more closely simulating the site. Columns can be planted for phytodegradation studies or substances added to evaluate competition or enhanced degradation rates. Unamended ambient controls can produce information on natural rates of degradation.

5.4.3 Confirmational Field Evaluations

5.4.3.1 In Situ Columns

The in situ column method can be used in portions of the perchlorate plume where there is reasonable expectation that natural attenuation is occurring. Using this procedure, Borden et al. (1997a) showed that decay rates measured in the in situ columns provided a better match with plume-scale degradation rates than conventional laboratory microcosms.

During the in situ biodegradation studies, columns of soil and groundwater, representative of the aquifer of concern, are isolated from the rest of the formation. The soil and groundwater within the columns are then treated with perchlorate so that a known concentration of perchlorate, and potentially the rate of perchlorate biodegradation, can be monitored over time within a controlled environment. Biodegradation rates obtained from the in situ biodegradation study may provide another line of evidence with which to evaluate perchlorate MNA.

5.4.3.2 Stable Isotopes

Monitoring isotopic ratios has promise of being a useful tool to measure the extent of perchlorate degradation. Stable isotope analysis provides a method for distinguishing biotic from abiotic attenuation. Microorganisms often preferentially use lighter isotopes in their metabolic processes (Mariotti et al. 1981; Heaton, 1986), and, as a contaminant is degraded, the isotopic composition of the remaining material becomes progressively heavier.

To use stable isotope methods to evaluate perchlorate attenuation at a site, groundwater samples should be collected and assayed for $\delta^{37}\text{Cl}$ of perchlorate. The sampling locations should include locations where groundwater conditions suggest that perchlorate may be biodegrading as well as background locations for comparison. The data are used to assess spatial variations in $\delta^{37}\text{Cl}$ to determine if there is significant isotopic fractionation during downgradient transport. If there is clear evidence of isotopic shifts, the extent of perchlorate degradation can be estimated using the fractionation factor. Degradation supported by isotopic measurements provides incontrovertible evidence that perchlorate is biodegrading and MNA is acceptable (Hatzinger et al., 2007).

6.0 SUMMARY

MNA of many contaminants has been shown to be a safe and cost-effective technology for remediating groundwater. However, each situation must be evaluated critically and the site-specific conditions considered separately before applying the technology. The guidance for addressing most MNA sites is a result of understanding gained from years of study on both the fate and transport of the many organic solvents that have been released into the environment. By contrast, perchlorate is an inorganic anion of a salt, meaning its behavior in the environment is vastly different.

The extent of perchlorate in the environment is becoming more widely acknowledged and its fate and transport are still being studied. Because of the potential health risks associated with its consumption, there is regulatory pressure to establish meaningful and realistic goals for cleanup. As more research is performed, both the regulated and regulatory communities will gain confidence that MNA of perchlorate can be a useful and reliable tool in many groundwater situations.

This document has been prepared to offer the reader guidance on how to approach assessing the potential for natural attenuation of perchlorate. In doing so, it provides information on the use of traditional, innovative and new tools for measuring perchlorate in the environment, and describes a tiered approach for obtaining data to support the conclusion that MNA is occurring. Not all tests need to be performed at all sites, but the tiered approach is a means to develop lines of evidence for the natural attenuation of perchlorate. If systematically and properly applied, MNA of perchlorate and can be relied upon to be protective of human and environmental health with just as much confidence as many other more costly groundwater remedies.

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APPENDIX A

POINTS OF CONTACT

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APPENDIX B

CASE STUDY 1 NAVAL SURFACE WARFARE CENTER, INDIAN HEAD, MARYLAND

**A Case Study for Perchlorate MNA at Indian Head, Maryland
Solutions-IES, Inc., Raleigh, NC 27607
ESTCP ER-0428**

Background

This case study site is located within the NSWC near Indian Head, Maryland, approximately 30 miles south of Washington, D.C. The Indian Head site consists of approximately 2 acres of grassy land containing a small drum storage building (Building 1419) and numerous groundwater MWs. Building 1419 was once used to clean out or “hog out” solid propellant containing ammonium perchlorate from various devices, including rockets and ejection seat motors, that had exceeded their useful life span. The hog out process and former waste handling methods impacted the groundwater with elevated concentrations of perchlorate. The groundwater flow direction suggests that perchlorate-contaminated groundwater migrates approximately 400 ft until reaching Mattawoman Creek, a large, tidally influenced tributary of the Potomac River.

To evaluate the use of MNA of perchlorate as a groundwater remedy at the Indian Head site, a tiered approach was used. This approach is similar to that used to evaluate MNA of VOCs.

Application of MNA Evaluation

Tier 1 – Perchlorate Plume Stability and Geometry

At the onset of the evaluation, a MW network was already in place at the Indian Head site. The well network had been installed to monitor the extent of perchlorate contamination and evaluate a pilot test of enhanced in situ bioremediation initiated in 2002 by Shaw Environmental near Building 1419. The prior work indicated that perchlorate concentrations decreased with distance away from the presumed source at Building 1419. However, perchlorate was not monitored beyond the pilot test area, which was located midway between the presumed source area and Mattawoman Creek, where the perchlorate plume was expected to discharge.

In 2005, with funding by ESTCP (Project No. ER-0428), Solutions-IES commenced its evaluation of the potential for MNA at the site. After baseline monitoring was performed, it became apparent that additional MWs and piezometer installations would be required to fully assess the plume geometry, including areas closer to the creek. Additional MWs and piezometers were installed in three portions of the site: 1) on land downgradient of the source area and closer to the creek, 2) in the intertidal zone and mudflat area (subtidal shallows) along the bank of Mattawoman Creek, and 3) a subtidal channel located between the intertidal zone and mudflats.

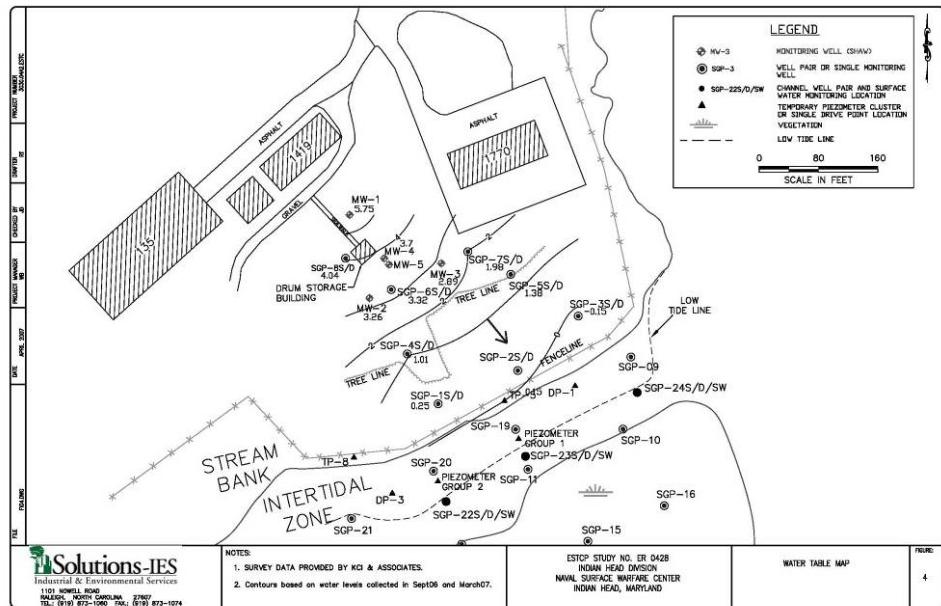


Figure 1. Site Map Showing Locations of Monitor Wells and Piezometers Showing General Groundwater Flow Direction Toward Mattawoman Creek.



Figure 2. View of the Tidal Flats in Winter.

At high tide, water flows downward into the aquifer from the creek. At low tide, groundwater flows upward through the organic-rich sediments before discharging to the surface as a series of small springs and seeps. The groundwater discharge area occurs primarily in an intertidal area adjoining a small subtidal channel.

Figure 1 shows the monitor well and piezometer network that was used in this evaluation and a general groundwater flow direction. Water level information and analytical results gathered during the Tier 1 evaluation indicated that perchlorate-laden groundwater flows to the southeast from the source area near Building 1419, eventually rising up through an intertidal zone, and ultimately discharging to Mattawoman Creek. The groundwater flow direction varies daily and seasonally according to tide levels in the freshwater creek. Figure 2 shows the tidal flats in winter when vegetation has died back, Figure 3 shows the same general area in summer.



Figure 3. View of Intertidal Channel in Summer.

Concentrations as high as 93,000 µg/L were measured near Building 1419; concentrations 400 ft downgradient beneath the bank of the creek remain over 10,000 µg/L. By extending the well network into the tidal flats and monitoring at different depths, cross-sections of the intertidal zone and perchlorate distribution were constructed that showed concentrations decreasing by over 99% as groundwater migrates upward through the organic rich sediments near the creek. However, because of the complicated groundwater flow regime, groundwater monitoring alone could not demonstrate that observed decline in perchlorate was solely due to biodegradation.

Tier 2- Biogeochemical Conditions for Perchlorate Biodegradation

Site-specific biogeochemical monitoring is the best understood and most widely employed evidence of perchlorate MNA. Concurrent with the Tier 1 evaluation, bio-geochemical parameters, including DO, ORP, TOC, methane, nitrate, sulfate, temperature, and pH were monitored to help determine if conditions within the groundwater at the Indian Head site were conducive to perchlorate biodegradation. Detailed monitoring of biogeochemical conditions within the intertidal zone showed that TOC and methane concentrations increase and ORP measurements decrease as groundwater migrates upward through organic-rich sediments in this area (Figure 4). These changes occur at the same depth that perchlorate concentrations decrease, providing supporting but indirect evidence of perchlorate biodegradation.

Although Tier 1 and Tier 2 data suggested strongly that perchlorate was attenuating naturally, additional evidence was needed to show more conclusively that this was occurring and to attribute the mechanism to biodegradation of the contaminant.

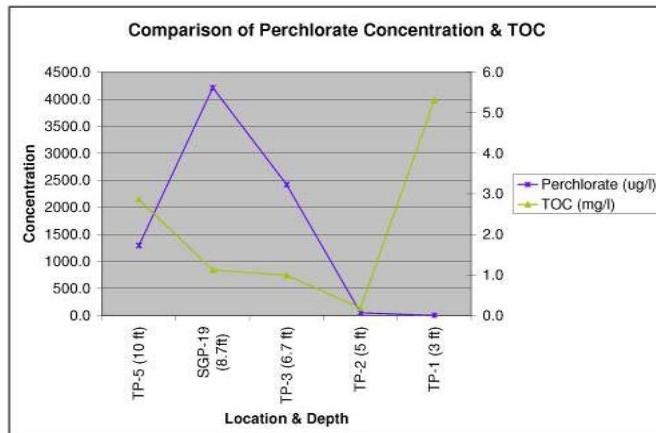


Figure 4. Comparison of Perchlorate and TOC Concentrations in Wells Located in the Tidal Flats.

Tier 3-Microbiological Indicators of Perchlorate Biodegradation

To provide direct evidence of the biological component of perchlorate biodegradation, various laboratory and field tests were employed. Both microcosm and macrocosm incubations were set up using site soil and groundwater. The microcosm setup utilized soil and groundwater from MW-2, a MW located close to Building 1419. The macrocosm setup utilized soil from the intertidal zone and groundwater from a well located near the edge of the intertidal zone. In the microcosms, perchlorate was reduced to below detection limits in less than 60 days, while the macrocosms showed at least a 40% reduction in perchlorate in less than 15 days.

Enzyme studies were also used. During the initial investigation at the Indian Head site, groundwater collected from MW-2 showed the presence of the CD gene, which mediates dismutation of chlorite, the final step in reduction of perchlorate to chloride and oxygen. This indicated that the capability to biodegrade perchlorate was present in indigenous microbial communities in groundwater at the site, but did not indicate the activity of the enzyme in situ.

Molecular analysis showed that high numbers of DNA-based *pcrA* genes occur in the intertidal zone. *PcrA* is involved in the degradation of perchlorate to chlorate and chlorite. Figure 5 shows the detected number of *pcrA* gene copies and the corresponding perchlorate concentrations in 17 wells/piezometers located along the intertidal channel. The data indicate that, in general, higher numbers of gene copies were reported in locations with lower perchlorate concentrations, suggesting that perchlorate is biodegrading as a result of *pcr*.

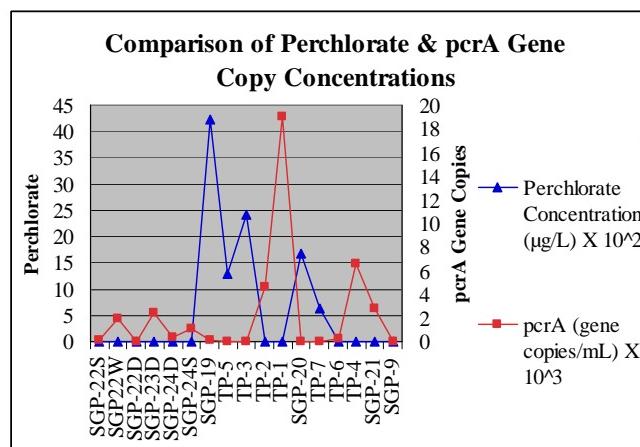


Figure 5. Comparison of *pcrA* Gene Copies and Perchlorate Concentration.

In situ field tests were also used to track reduction in perchlorate due to biodegradation. At the Indian Head site, in situ columns were installed within the intertidal zone to provide a direct measure of bioactivity. The in situ columns were constructed such that a column of soil within the intertidal zone is isolated from the surrounding soil and water with an open ended PVC pipe (Figure 6). Groundwater is slowly pumped upward through the column at rates comparable to the natural groundwater flow velocity. First-order biodegradation rates were estimated that range from 24 to 61 per year.



Figure 6. View of In Situ Columns Installed in Tidal Flats with Adjacent Piezometers.

Summary

A tiered approach was employed to demonstrate MNA of perchlorate at the Indian Head site. Tier 1 results showed that perchlorate concentrations slowly decrease as the groundwater moves away from the source and rapidly decrease as the contaminant moves vertically through the organic rich intertidal zone near Mattawoman Creek. Tier 2 results showed that the rapid decline in perchlorate concentration within the intertidal zone occurred at the same depth that TOC and methane increased, and ORP decreased, providing supporting but indirect evidence of perchlorate biodegradation. Together, the plume configuration suggested that several controlling factors including dilution, dispersion and biodegradation were responsible for the observed attenuation of the contaminant.

Microcosm and macrocosm incubations (Tier 3) constructed with groundwater and soil from the Indian Head site demonstrated perchlorate biodegradation in a short period of time. DNA enzyme assays performed on groundwater samples collected within the intertidal zone show that high numbers of *pcrA* genes occur where perchlorate concentrations decline. In situ columns, installed in the stream bed to provide a direct measure of in situ biodegradation, measured first-order biodegradation rates from 24 to 61 per year.

The trends in groundwater flow, biogeochemical parameters, microbial populations and perchlorate concentrations provide multiple lines of evidence that perchlorate is biodegrading at the Indian Head site prior to discharge to Mattawoman Creek. The findings, when considered together, could be used to form the basis of a recommendation that perchlorate MNA may be an acceptable remedy at this site.

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APPENDIX C

CASE STUDY 2 ALLIANT TECHSYSTEMS, LLC, ELKTON, MARYLAND

A Case Study for Perchlorate MNA at Elkton, Maryland Solutions-IES, Inc. ESTCP ER-0428

Introduction

This case study site is located at the ATK facility, approximately 60 miles northeast of Baltimore near Elkton in Cecil County, Maryland. The ATK facility has been used for multiple industrial purposes, such as fireworks manufacturing, munitions production, pesticide production, and research and manufacturing of solid propellant rockets. Ammonium perchlorate was used in rocket engine testing and manufacturing at the facility. Soil and groundwater investigations were initiated during the 1980s when TCE was detected in two production wells at the facility. The area of focus for these investigations is designated the Perchlorate/TCE Solid Waste Management Unit (SWMU) (the site).

To demonstrate the use of MNA of perchlorate at the ATK site as a groundwater remedy, a tiered approach was adapted: Tier 1—determine the spatial and temporal distribution of perchlorate; Tier 2—characterize the suitability of biogeochemical conditions for perchlorate biodegradation; and Tier 3—confirm microbiological indicators of perchlorate biodegradation.

Application of MNA Evaluation Approach

Tier 1 – Perchlorate Plume Stability and Geometry

Most of the perchlorate contamination within the Perchlorate/TCE SWMU was previously defined using a network of MWs screened in the shallow, intermediate, and deep aquifers. The perchlorate contamination is largely confined to the shallow and intermediate aquifer within the Perchlorate/TCE SWMU. The highest concentrations of perchlorate detected are in the vicinity of the SWMU, with concentrations in this area as high as 1,030 µg/L. The perchlorate and TCE groundwater plume extends from west to east beyond the ATK property, approximately 3,000 ft from the presumed source. The distal extent appears to be limited by interception at Little Elk Creek (Figure 1). Data obtained during routine monitoring of the site indicate that perchlorate is below detection limits east of Little Elk Creek. However, some TCE has migrated beyond the creek suggesting the possibility that perchlorate may have attenuated prior to discharging to the creek.

After baseline monitoring was performed for the demonstration in 2005, additional MWs were installed to fill out the network and help assess plume geometry. These MWs were located just east of Elkton Road (SMW-9S/M, SMW-13S/M, and SMW-11S/M), and north of the SWMU area (SMW-8S/M). Figure 1 shows MW locations and isoconcentration contours of perchlorate in the intermediate aquifer derived from the baseline sampling results.

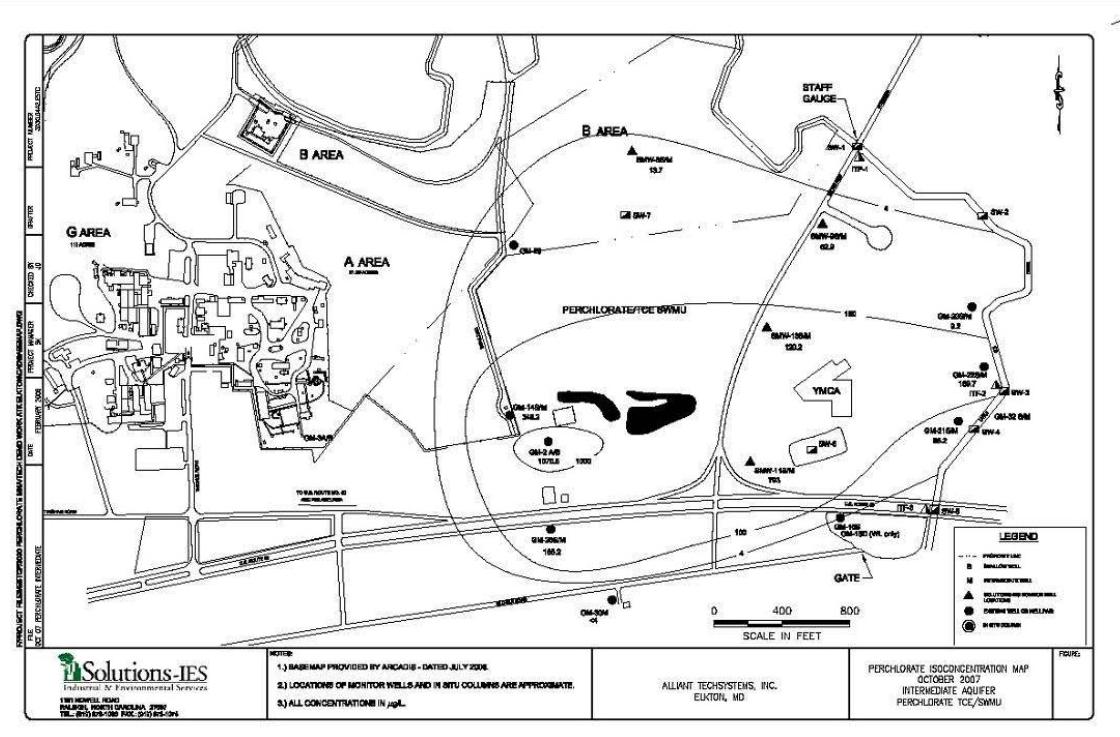


Figure 1. Site Map Showing Shallow and Intermediate-Depth Monitoring Wells and the Intermediate Perchlorate Isoconcentration Contours.

Each of the MW pairs included a MW in the shallow aquifer installed to a depth of approximately 30 ft bgs, and a MW in the intermediate aquifer at a depth of approximately 60 ft bgs.

Water level information and analytical results gathered from this Tier 1 evaluation indicate that groundwater typically flows to the east prior to discharge to Little Elk Creek. Little Elk Creek is a shallow stream that traverses a zone of undeveloped land covered with shrubs, vines, and trees. The width of the naturally occurring buffer on the west side of the creek is approximately 50 ft including the stream bank which is an alluvium deposit composed of sand and gravel.

Groundwater analytical results utilized as part of the Tier 1 evaluation indicated that perchlorate tends to concentrate in the intermediate aquifer as groundwater flows to Little Elk Creek. Figure 2 shows perchlorate sampling activities near Little Elk Creek. However, as perchlorate nears Little Elk Creek, the intermediate aquifer thins, and the perchlorate concentrations tend to increase as it begins to flow through the



Figure 2. Sample Collection Along the Shore of Little Elk Creek Using the Filter Stack Method for Perchlorate.

shallow aquifer. Groundwater analytical results indicated that the plume geometry had changed very little since Solutions-IES began monitoring for this demonstration project in 2006 and that the plume is generally stable.

Tier 2- Biogeochemical Conditions for Perchlorate Biodegradation

The collection of site-specific biogeochemical information is the best understood and most widely employed step to provide supporting evidence of MNA of perchlorate. Concurrent with the Tier 1 evaluation, biogeochemical parameters such as DO, ORP, TOC, methane, nitrate, chloride, temperature, and pH were monitored to help determine if conditions within the groundwater at the ATK site, especially near the discharge point at Little Elk Creek, were conducive to the biodegradation of perchlorate. DO concentrations near Little Elk Creek historically were below 2.5 ppm with some locations closer to 1 ppm; ORP measurements were generally less than +60 mV. These conditions are not optimal for perchlorate reduction but may still support the growth and activity of perchlorate-reducing microorganisms.

Tier 3-Microbiological Indicators of Perchlorate Biodegradation

To provide direct evidence of the biological component of perchlorate biodegradation, both laboratory and field tests were performed at or on matrices from the ATK site. Laboratory microcosms were set up utilizing sediment and groundwater from GM-22S, a MW located close to Little Elk Creek along the plume centerline. The microcosm results showed a reduction in low starting concentrations of perchlorate under ambient conditions to detection limits in about 120 days and a zero-order degradation rate of 0.92 mg/L/yr (Figure 3).

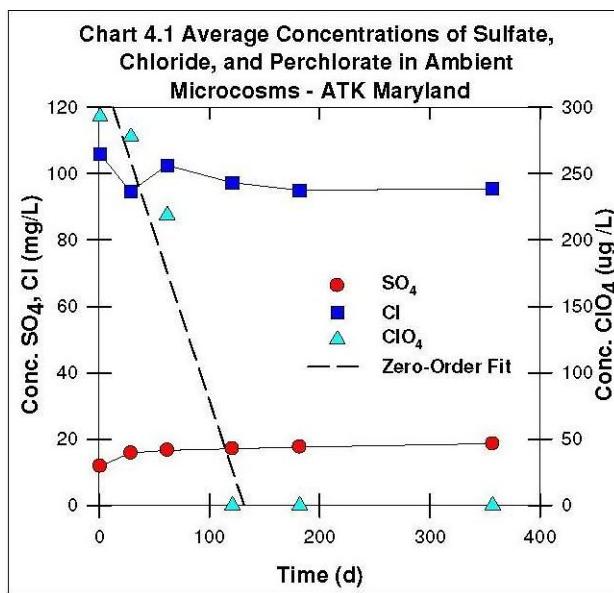


Figure 3. Biological Reduction of Perchlorate in Ambient Microcosm Treatments.

Other laboratory testing for direct evidence biodegradation of perchlorate was performed on a soil boring sample of sediment near Little Elk Creek. The CD enzyme assay showed a positive genetic potential to produce CD and, therefore, a potential to degrade perchlorate under the appropriate conditions.

The DNA-based qPCR *pcrA* gene assay was also utilized at the site to determine if the genetic potential is present to biodegrade perchlorate. *PcrA* gene copies were detected in four of the 23 groundwater samples collected. Of these four samples, two were collected from the shallow aquifer in MWs located along the Little Elk Creek, suggesting that perchlorate may be biodegrading in the riparian buffer zone along Little Elk Creek.

In addition to laboratory tests, field tests were also utilized to help identify perchlorate biodegradation. Three in situ columns were installed near the creek bed close to the center line of the perchlorate plume (GM-22 S/M). Two of the columns were live biotic columns, and the other column was inhibited with nitric acid. The design of the columns is similar to the system used by Gillham et al. (1990) and Borden et al. (1997a) and consists of a 3-foot long stainless steel test chamber, allowing sediment and groundwater to be isolated from the surrounding environment (Figure 4a). Tubing is attached to the top of each column to allow for the injection of a known concentration of perchlorate into each column, and as a sampling port (Figure 4b). Each of the columns was injected with perchlorate at a concentration of approximately 150 µg/L in June 2007. After monitoring groundwater samples collected from the in situ columns over a period of approximately 6 months, perchlorate decreased from 133 µg/L to below the analytical detection limit with first-order biodegradation rates ranging from seven to nine per year (Figure 5).

Figure 4. Schematic of In Situ Column Design.

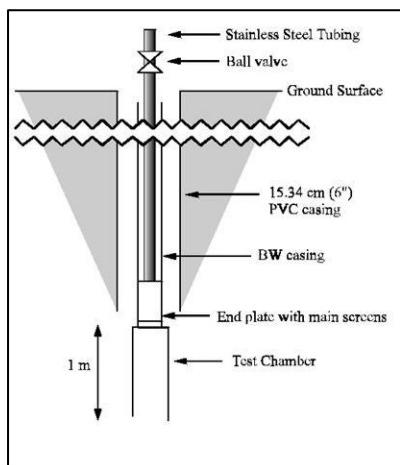


Figure 4a. In Situ Column Construction.



Figure 4b. Photograph of In Situ Columns in Place.

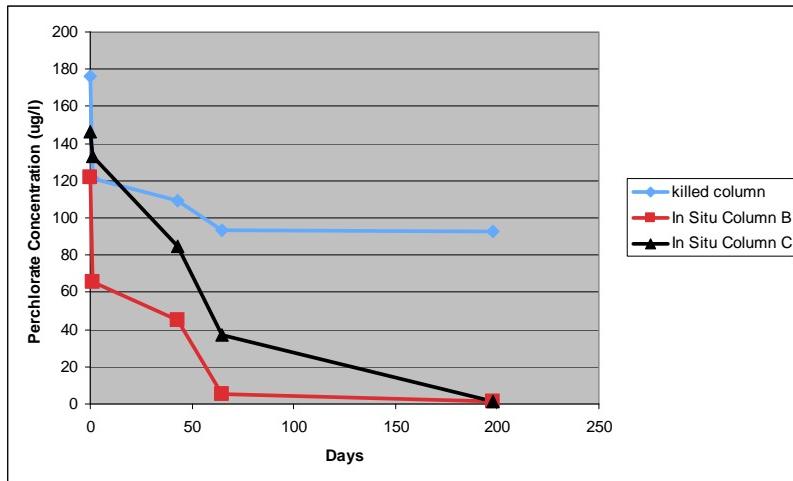


Figure 5. Decline in Perchlorate Concentration Versus Time in Live Columns (B and C) Compared to No Degradation in Killed Column.

Summary:

The tiered approach was employed to document the MNA of perchlorate. In Tier 1 of the evaluation, the perchlorate plume was shown to be stable with evidence that perchlorate may be degrading near Little Elk Creek. The Tier 2 evaluation of biogeochemical parameters indicated that conditions were not optimal but were adequate to support natural attenuation near the creek.

The Tier 1 and Tier 2 results alone were not sufficient to thoroughly document MNA so additional Tier 3 testing was conducted to demonstrate perchlorate biodegradation. In laboratory microcosms constructed with groundwater and soil from near the creek, perchlorate was biodegraded to below the analytical detection limit in less than 120 days. DNA enzyme assays performed on groundwater samples demonstrated that microorganisms capable of perchlorate biodegradation are present in the aquifer. Gene copies associated with the enzyme used to degrade perchlorate were highest in the riparian buffer near the creek. Field column tests demonstrated in situ biodegradation of perchlorate to below detection 200 days in with a first-order biodegradation rate of seven to nine per year.

The case study at the ATK site illustrates the use of the tiered approach for demonstrating MNA of perchlorate in groundwater. These findings could be used to support the use of MNA as the recommended remedy at this site.